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# MASTERARBEIT

Titel der Masterarbeit

## Growth rates of Chrysophytes in dependence on light intensity and bacteria abundance


verfasst von

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# Table of Contents

# 1. Introduction

Mixotrophy is the ability of phytoplankton organisms to gain nutrients and energy by the combination of photosynthesis and phagotrophy. A mixotrophic organism combines therefore two modes of nutrition, which otherwise are found in different organisms (plants and animals). Some scientists include to the term mixotrophy the ability of taking up dissolved organic compounds (osmotrophy) instead of bacteria ingestion. (Flynn et al. 2012) However, probably all phytoplankton organisms are osmotrophs, so here I want to refer to mixotrophs as protists that combine photosynthesis and bacterivory.
Mixotrophy is not limited to phytoplankton, but is also found in other protists like ciliates and even in metazoans, e.g. some sacoglossan molluscs. Those are able to store chlorophyll from ingested phototrophic organisms and use it for their own metabolism. (Raven et al. 2009, Raven 1997)

Phototrophy in eukaryotes is a derived characteristic as the first eukaryotes were all phagotrophic. They gained the ability of photosynthesis by ingesting cyanobacteria as endosymbionts. In the course of evolution these cyanobacteria lost the genes needed for their independent existence, while most of the genes necessary for photosynthesis were transferred to the cell nucleus of the host. Purely phototrophic eukaryotes lost the ability to ingest bacteria or other prey while mixotrophs kept both modes of nutrition. (Raven 1997)

Traditionally, plankton species were categorized as either phytoplankton or protozooplankton. At the same time these terms divided plankton organisms into either primary or secondary producers. However, evidence suggests that many protists in illuminated waters are mixotrophic and contribute to both primary and secondary production at the same time. Therefore, the role of phytoplankton in aquatic ecosystems should be reconsidered. (Flynn et al. 2012)

Mixotrophs can become very abundant or even dominant in surface waters in lakes during summer. In temperate climate zones after water turnover at the beginning of the year, nutrients are well distributed. These conditions favour specialized phototrophs or phagotrophs, which are considered r-strategists. In summer lakes become stratified which prevents transport of nutrient rich deep water to the surface. As a result, surface waters experience nutrient depletion which favours mixotrophs that are considered to be K-strategists. (Mitra et al. 2014)

The strategy of mixotrophy is especially advantageous when resources are limiting. (Jones 2000, Rothhaupt 1996b) Often chrysophytes are dominant in oligotrophic or humic lakes. In humic lakes light and concentration of dissolved organic nutrients are low, while the high concentration of

dissolved organic matter leads to a high bacterial abundance. (Jones, 2000) Phytoplankton species in oligotrophic lakes use mixotrophy as a strategy to compensate for low concentrations of nutrients like phosphorus, nitrogen and iron. They are able to complementarily use light, dissolved nutrients and nutrients from bacterivory. (Raven 1997, Flynn et al. 2012).

However, mixotrophic strategies also come with costs: The organisms have to maintain cell structures for photosynthetic and phagotrophic nutrition. According to estimates by Raven (1997), maintenance of the photosynthetic apparatus can account for up to 50% of the energy and nutrient costs of cell synthesis in a phototrophic organism. In contrast, the costs for the phagotrophic apparatus account for <10% of energy and nutrients. This trade-off leads to reduced resource use efficiency in mixotrophs and results in lower maximum growth rates compared to specialized phototrophic or heterotrophic organisms. (Katechakis and Stibor 2006)

Mixotrophs compete with phototrophs for light and inorganic nutrients and with heterotrophs for particulate organic nutrients. (Pålsson and Daniel 2004) Besides, bacteria are stronger competitors than mixotrophs regarding inorganic nutrient uptake like phosphorus. Therefore, bacterivory is beneficial in two ways: Bacteria are a P source for mixotrophs, especially when phosphorus is limiting (Nygaard and Tobiesen 1993) and at the same time they reduce their competitor for inorganic nutrients. (Thingstad et al. 1996)
In oligotrophic environments mixotrophs are usually found in surface waters. They lower bacteria density to the phagotrophs' critical food concentration so the latter are not able to sustain in the ecosystem. (Tittel et al. 2003; Hartmann et al. 2012)

In a study regarding marine planktonic protists, scientists found out that plastidic and aplastidic protist populations exhibit comparable cumulative bacterivory rates. Plastidic protists have lower cellular bacterivory rates but are much more abundant than aplasitdic protists. In line with that, they may account for 40-95% of total bacterivory in oligotrophic waters. (Zubkov and Tarran 2008) Approximately half of the total phytoflagellate biomass in oligotrophic coastal waters consists of three mixotrophic groups. They are responsible for 9-42% of total bacterivory in the ecosystem. (Unrein et al. 2013)

In oligotrophic oceanic gyres small plastidic protists, comprising e.g. several species of *Chrysophyceae* and *Prymnesiophyceae*, are the most abundant group of eukaryotes. Although they are outcompeted by the more abundant bacterioplankton for the uptake of phosphate they are major contributors to $CO_2$ fixation. Thus, Hartmann et al. (2011) suggested that plastidic protists compensate the limited

access to inorganic nutrients by mixotrophy. This implies that phytoplankton is not exclusively dependent on dissolved inorganic nutrients, as previously thought. Because nutrients are scarce but light energy is plentiful, surface waters of oligotrophic oceanic gyres are the ideal habitat for mixotrophs. (Hartmann et al. 2012)

Detailed studies on growth kinetics, especially the influence of light on mixotrophic growth, are scarce. Growth rates of *Poterioochromonas malhamensis* were studied in several experiments. Light intensity and bacteria concentration varied and algae were also kept in complete darkness. (Rothhaupt 1996a and 1996b, Pålsson and Daniel 2004) Due to the lack of parameters, we are unable to represent mixotrophs in models of the micorobial loop, though mixotrophs are often the dominant bacterivores in oligotrophic systems (Mitra et al. 2013). Likewise, we lack empirical data to quantify mixotrophy as a specific trait. Data on mixotrophic growth kinetics are also required for trait-based understanding of microbial food webs. (Litchman and Klausmeier 2008)

### 1.1 Community Experiment

Mixotrophs compete with phototrophs for light and nutrients and with heterotrophic protists for prey. Growth kinetics of an obligate heterotroph (*Spumella sp*.) and a mixotroph (*Poterioochromonas malhamensis*) were compared in an experiment (Rothhaupt 1996b). The mixotroph could supplement energy use from photosynthesis by phagotrophy, hence the prey concentration required for zero net growth depended on light intensity (Figure 1). Based on these observations, Tittel et al. (2003) proposed the mechanistic resource competition theory.

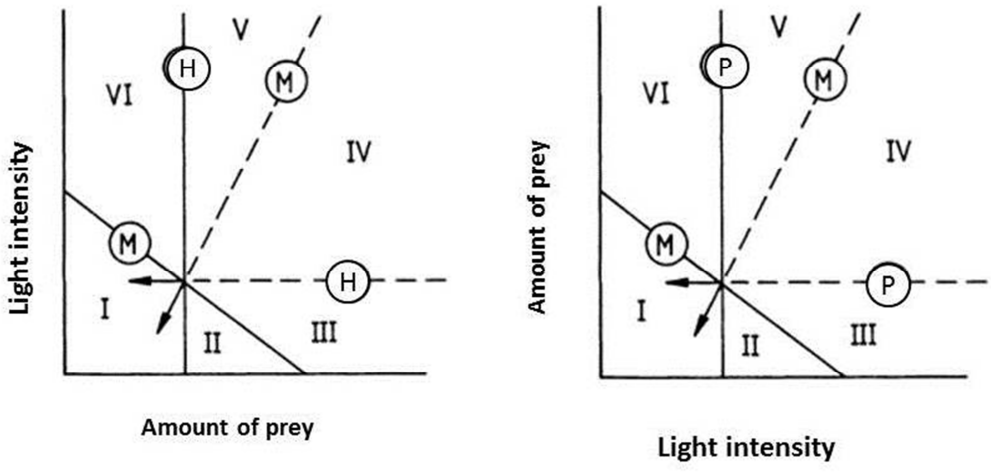



**Figure 1**The zero net growth isoclines in the resource plane indicate resource concentration for mixotrophs (M) and heterotrophic or photoautotrophic specialized organisms (H and P) at which reproduction and losses are in equilibrium. (modified figure from Rothhaupt 1996b)

For *P. malhamensis* experiments revealed a release of soluble reactive phosphorus (SRP) in the dark or at high bacterial densities. When growing primarily phototrophic, the organism took up SRP. Therefore, depending on the prevailing nutritional strategy, mixotrophs can either facilitate nutrient-limited organisms or compete with them. (Rothhaupt 1997, 1996a, 1996b)
Depending on the amount of resources in the ecosystem and on the algae's requirements, the mixotrophs might outcompete the phototrophs or coexist with them. (Rothhaupt 1996b, Jones 2000)

These assumptions lead to predictions about the outcome of competition. Before starting the experiments, data from Lake Lunz were investigated, regarding the distribution of light and temperature (Figure 3) and the abundance of *Dinobryon divergens* and A*sterionella formosa* (Figure 2). (Data were collected during a course of the University of Vienna in summer 2014)

On June 17, 2014, at 11:30 AM, which was a sunny day, light intensity was 570µEm$^{-2}$s$^{-1}$ at 1m depth and decreased exponentially with depth. The thermocline was located at a depth of 5m, where light intensity was 80 µEm$^{-2}$s$^{-1}$ and the abundance of *Asterionella* was highest. (Figure 3) *Dinobryon* was more abundant than *Asterionella* at the surface of the lake but decreased rapidly down the water column.

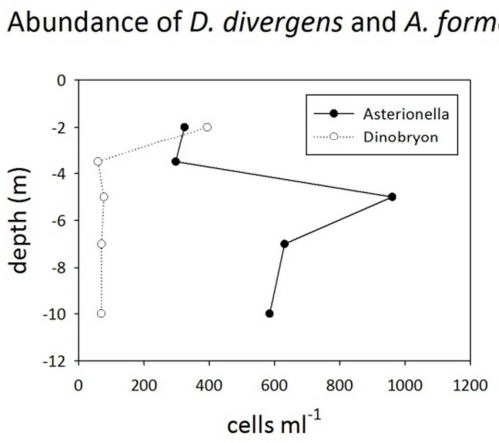



**Figure 2** Abundance of *Dinobryon divergens* and *Asterionella formosa* in Lake Lunz. Data was collected in June 2014.

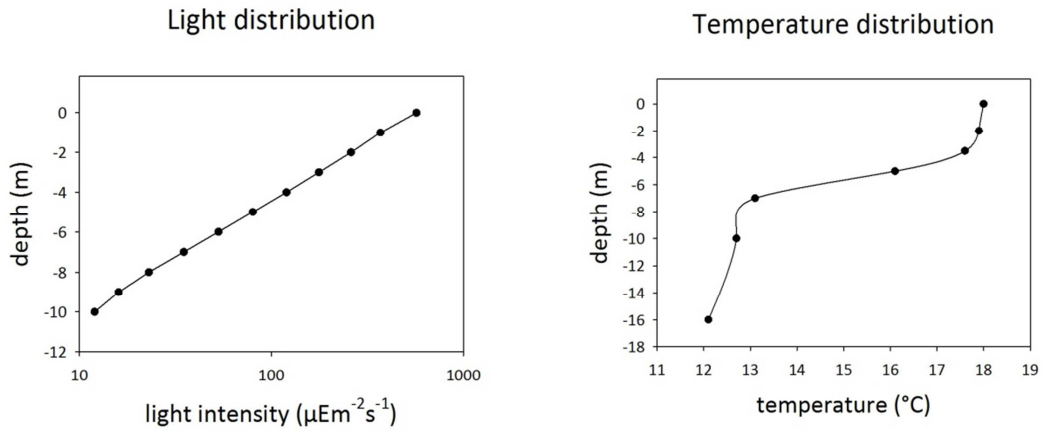



**Figure 3** Distribution of temperature and light in Lake Lunz in June 2014.

For community experiments these two species were exposed to a threefold light gradient. Results of the laboratory experiments were compared to data collected from Lake Lunz.

### 1.2 Aims and Hypotheses

The aim of the experiments was to characterize growth kinetics of the three chrysophyte species *Dinobryon divergens*, *Ochromonas tuberculata* and *Poterioochromonas malhamensis* and to compare them to photoautotrophic algae.

In this context, thresholds of prey abundance and light availability for growth of mixotrophic populations play an important role. For example, saturating light levels of different species and the amount of prey they need, can lead to predictions where in the water column they have their ecological niche and give insight into their function in nutrient cycling.

The experiments were conducted under different light conditions. In a second treatment the mixotrophs were examined at elevated bacteria densities.

The community experiment was also conducted under a light gradient to find out at what light conditions the mixotroph is able to coexist with or even outcompete the photoautotrophic protists.

Based on information from the literature and from the results of experiments done before, the following hypotheses were tested:

I. Mixotrophs have got lower maximum growth rates compared to obligate phototrophic species.
II. Higher bacterial abundance will lead to higher maximum growth rates in the mixotrophs due to additional uptake of nutrients.

III. Light saturation is reached at higher light intensities by mixotrophs than by photoautotrophs.

IV. Mixotrophs accommodate different cell structure and therefore will have less chlorophyll-a per unit biovolume than photoautotrophs.

V. At high light conditions the mixotrophic alga will coexist with the phototroph. The phototroph will be more efficient when light is limiting and will outcompete the mixotroph.

VI. Presence of mixotrophic bacterivores in a community will lead to a reduction of bacteria abundance as compared to a community that only contains heterotrophic bacterivores.

# 2. Materials and Methods

### 2.1 Monoculture Growth Experiments

A series of growth experiments was conducted with three mixotrophic phytoplankton species: *Dinobryon divergens*, *Ochromonas tuberculata* and *Poterioochromonas malhamensis*. *D. divergens* has been isolated from Lake Lunz a year before and *O*. *tuberculata* and *P*. *malhamensis* were obtained from the culture collection of the Aquatic Ecology group at the University of Munich (Prof. Stibor). The *P. malhamensis* strain is identical to the culture used by K.O. Rothhaupt (called *Ochromonas sp.* in his papers, 1996 a and b, 1997)

Experimental design

All algae were grown as batch cultures on WC medium (after Guillard and Lorenzen 1972), except for *D*. *divergens* which did not grow on WC medium. *D. divergens* grew on a medium consisting of 90% lake water plus 10% WC medium. (hereafter referred to as $SW_{10}$).

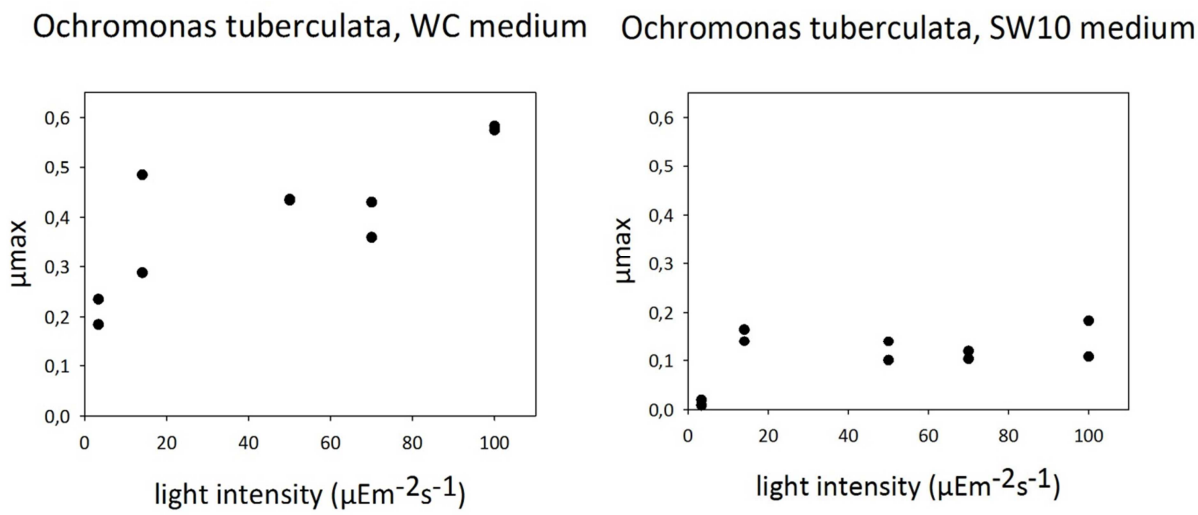



**Figure 4** *Ochromonas tuberculata* growing on $SW_{10}$ and WC medium. In both experiments glucose was added.

In the first experiments also *O. tuberculata* and *P. malhamensis* grew on $SW_{10}$ in order to use the same medium for every species. However, *O. tuberculata* had very low growth rates on $SW_{10}$ compared to WC medium, even with the addition of glucose. (Figure 4)

Cultures were exposed in replicates to the following light gradient: 100, 70, 50, 14 and 3 $\mu Em^{-2}s^{-1}$. This gradient was achieved by covering the culture flasks with neutral density filters transmitting corresponding percentages of light (70, 50, 14%, Lee Filters). (Figure 6) For some intensities, two filters were combined to achieve the desired shading. Cultures were kept in a climate chamber under a light:dark regime of 16:8h at 20°C.
Chlorophyll-a autofluorescence was measured on a fluorometer (450nm; AquaPen, YSI) and based on these data growth rates of algae were calculated.

To start the experiments, the ratio of inoculum:medium was chosen in that way, the starting values given by AquaPen were just above 100, i.e. highly diluted but in a measurable range. Two sizes of culture flasks were used: 100ml and 750ml. For the light levels 70 and 14 $\mu Em^{-2}s^{-1}$ large flasks were used with a starting volume of 400ml. At these two light levels samples were filtered over glass-fibre filters, so more volume was needed. The culture flasks at light levels 100, 50 and 3 $\mu Em^{-2}s^{-1}$ were small, containing 40ml.

In one of the experiments I noticed that *Ochromonas* tends to grow on the surface of the culture flasks, which could lead to an underestimation of maximum growth rates. (Figure 5) To avoid this bias, in the following experiments cell scrapers were used daily before sampling to remove cells attached to the walls of the culture flasks. The same effect was observed in the *Poterioochromonas malhamensis* cultures but not for *Dinobryon divergens*.

This 'wall growth' may be due to bacteria biofilms forming on the walls of the culture flasks, so the mixotrophic algae accumulate where prey is abundant.
Growth rates might seem to be reduced because more algae are located at the walls than in the medium so they are also not detected in the samples taken with the pipette. Besides, if algae are not able to graze the biofilms, increased bacteria abundance would lead to an increased bacterial uptake of nutrients. This could result in reduced algal growth rates due to reduced availability of nutrients.

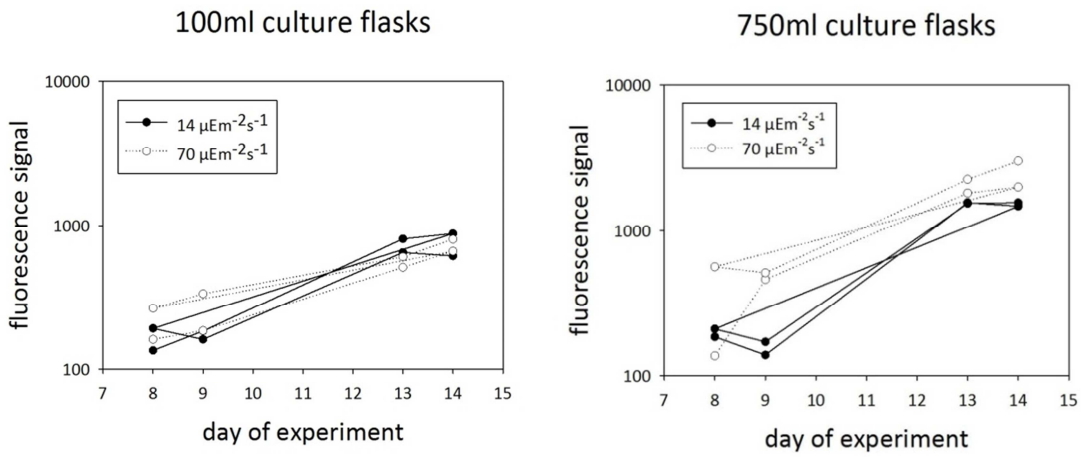



**Figure 5** Growth of *Ochromonas tuberculata* in 100ml and 750ml culture flasks at 70 and 14µEm-2s-1. In both experiments glucose was added.

In a second set of experiments bacterial growth was triggered by a daily addition of 10mgL$^{-1}$ of glucose. Higher bacterial abundance should facilitate growth of mixotrophic phytoplankton. (Rothhaupt 1996b, Ptacnik 2003) For this purpose a 40mmolar glucose solution was made and the amount of solution necessary for an end concentration of 10mgL$^{-1}$ was pipetted into the culture flasks daily, using a 100ml and a 1000ml pipette.

Phytoplankton organisms are able to use glucose as a nutrient themselves. To avoid higher algal growth rates as an effect of glucose addition, only a very small amount was added. Furthermore, bacteria are well known to take up nutrients much faster than bigger algae due to their higher surface to volume ratio. (Currie and Kalff 1987) Sanders et al. (1990) could demonstrate that addition of glucose itself does not increase growth rates of P. malhamensis when prey abundance is too low.

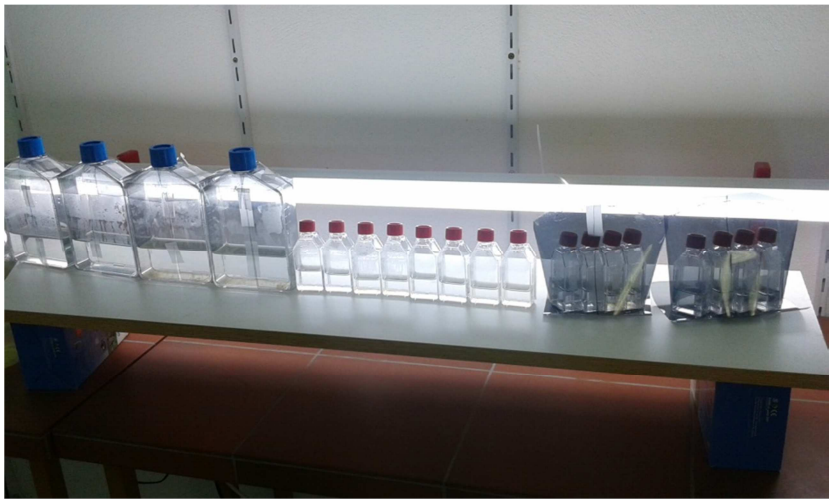


**Figure 6** Monoculture batch experiments under a light gradient that was achieved using neutral density filters

Sampling Methods

Samples for cell densities and autofluorescence were taken every other day over the course of the experiment using a 5ml pipette for the large flasks and a 1ml pipette for the small ones. Samples of 3ml were used to measure chlorophyll-a using AquaPen and based on these data growth rates were calculated.

For analysis of cellular nutrient concentrations and photometric analysis of chlorophyll-a, larger volumes were required. Twice during the experiment, during the exponential and stationary phase of growth, samples were taken from the large culture flasks and filtered onto precombusted and acid-washed glass-fibre filters (Whatman GF/F). Three filters were used, for analysis of chlorophyll-a content, particulate organic carbon/nitrogen (POC/PON) and particulate organic phosphorus (POP). For each of the filters a volume of 20-50ml was used. Filters were stored at -20°C until analysis. In addition, at the same time points, 2ml were fixed with 1% Lugol's Iodine solution for cell counting and calculation of biovolume.

Analyses

In a given time interval, growth rates were calculated based on the chlorophyll data measured with AquaPen using the following formula: $\ln(t_2/t_1)/d$. $t_1$ and $t_2$ are the autofluorescence measurements on the two time points, d is the number of days between these time points.

First, a suitable time-interval was selected by visually inspecting the growth curves (see Figure 7). In a semi-log plot, exponential growth appears as linear increase. For example, in Figure 7 exponential growth is seen at 100µEm$^{-2}$s$^{-1}$ from day 4 to 9. Hence, in this case the maximum growth rate for this time interval was calculated.

I also tried to determine maximum growth rates using a statistical model based on the assumption that logistic growth should follow an s-curve. However, this approach did not fit the empirical data very well. (Figure 7) Therefore, the method mentioned above was used.

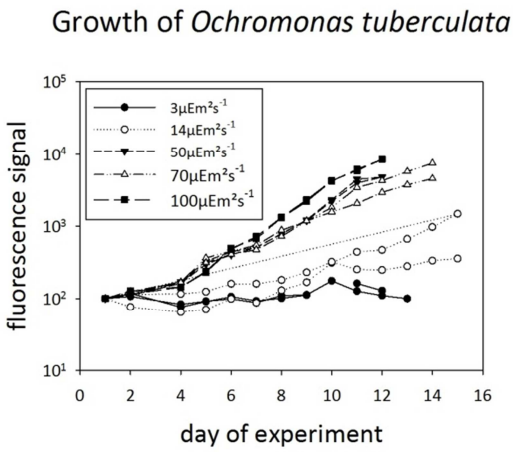



**Figure 7** Growth of *Ochromonas tuberculata* with glucose added to exemplify that at low light intensities growth does not follow an s-curve**.**

Cells were counted and measured on an inverted light microscope using settling chambers. Biomass was estimated by approximating species-specific cellular biovolume from simple geometric bodies. (Hillebrand 1999) Concentration of chlorophyll-a was determined in a fluorometer after extraction in acetone with quartz sand. For measuring POP concentration, filters were combusted at 550°C for 10 hours. After elution in 5ml of 4,5M $H_2SO_4$ samples were measured via molybdate reaction and photometric analysis after Grasshoff (1999).

Filters for POC/PON analysis were dried at 60°C, folded, packed in tin caps, and analysed by heat combustion in an organic elemental analyser (Thermo Scientific).

For statistical analysis of the data the free software RStudio was used. Plots were created using either RStudio or SigmaPlot.

### 2.2 Community Experiment

In the community experiment I tried to mimic the situation in Lake Lunz. To achieve this, a light gradient was established and $SW_{10}$ was used, as Lake Lunz is an oligotrophic lake.

The mixotroph *Dinobryon divergens* was grown in competition with the heterotrophic nanoflagellate (HNF) *Spumella sp*. and the photoautotroph *Asterionella formosa*. To test the hypothesis that the mixotroph is able to reduce prey in the established communities, a treatment without *Dinobryon* was run in parallel.

*Dinobryon divergens* and *Asterionella formosa* are both isolates from Lake Lunz. The three species were grown together in culture flasks over 16 days in a light gradient of 3 light intensities.

Experimental Design

The community experiment was conducted as an exponentially fed batch (EFB) culture on $SW_{10}$ medium. EFB compares to a chemostat, but has no outflow. Medium is added proportionally to current medium by a computer controlled peristaltic pump. (Fischer et al. 2014) The community in the 250ml culture flasks consisted of the mixotroph *D. divergens*, the phototroph *A. formosa* and the heterotrophic nanoflagellate (HNF) *Spumella sp.* Bacteria were present from non-axenic growth of the cultures. The HNF was added to mimic the natural situation where a mixotroph competes with both autotrophic and heterotrophic competitors. In a second set of culture flasks the same community was established without *D. divergens*, to compare its effect on the development of picoplankton populations.

Culture flasks were kept in replicates at three light levels which were achieved using neutral density filters: 100, 50 and 14µ$Em^{-2}s^{-1}$. Starting volume in the culture flasks was 20ml and the dilution rate for addition of medium was 0.2 $day^{-1}$. For addition of medium a peristaltic pump was controlled by a microcontroller (Arduino). (Figure 8) The program was set such that the inflow was always proportional to the current volume and was set back to the initial volume at each sampling.

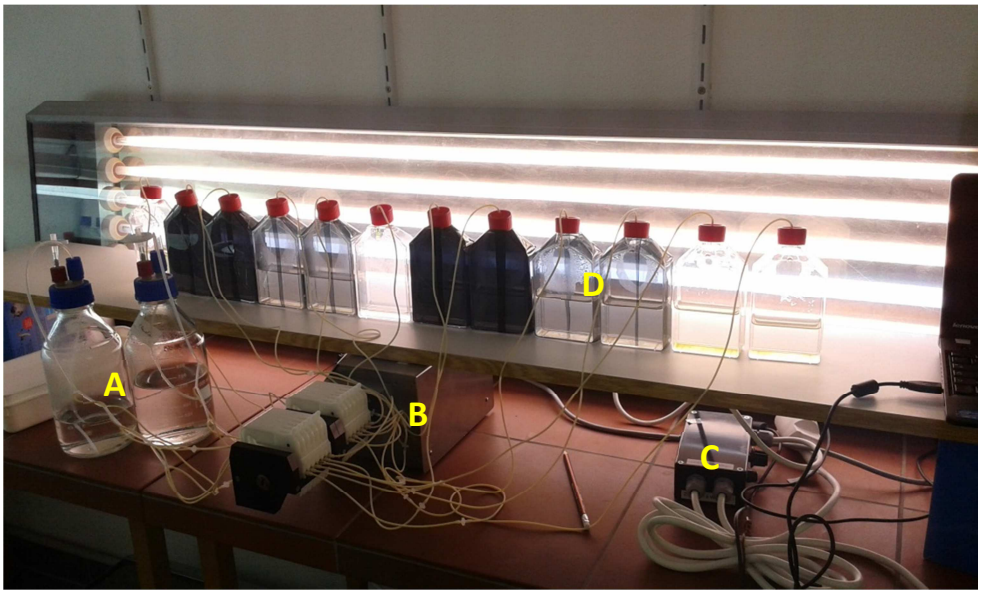



**Figure 8** Experimental setup of the competition experiment. A: medium bottles, B: peristaltic pump, C: microcontroller, D: culture flasks

Sampling Methods

Every two to three days samples of 3ml were taken and used for fluorometric measurement of chlorophyll-a, using AquaPen, and turbidity, using a photometer. These samples were then fixed with 1% Lugol's iodine solution for cell counting.

Turbidity was measured as a second method to determine growth rates of phytoplankton. However, compared to results from chlorophyll-a measurements, growth rates calculated from turbidity values were not as accurate. Therefore, I did not use them for further analysis.

After 6, 13 and 16 days additional samples of 3ml were taken for counting of bacteria. The samples were fixed with 0,5% Glutaraldehyde, quick-frozen in liquid nitrogen and stored at -80°C until analysed by a FlowCytometer.
On day 16 the experiment was terminated. Water samples from the culture flasks were filtered onto precombusted and acid-washed glass fibre filters (Whatman GF/F) for analysis of chlorophyll-a concentrations and POC, PON and POP content. Filters were stored at -20°C until they were analysed.

Analyses

For analysis of chlorophyll-a, POC, PON and POP the same methods were used as for monoculture growth experiments. Additionally, 2ml of each sample were counted on an inverted microscope for determination of cell concentrations and biovolume.
Glutaraldehyde fixed samples were stained with SYBR Green and analysed with a Flow Cytometer. (Brussaard 2004, Winter et al. 2009) Cell concentrations of bacteria in the samples were extracted using the free Software "Flowing Software". Unfortunately, the dot plots could not be gated, so for further calculations I used total counts of bacteria.

For statistical analyses and creating corresponding plots, RStudio and Sigmaplot were used.

# 3. Results

## 3.1 Monoculture Growth Experiments

Growth rates

Maximum growth rates were plotted in dependence on light intensity and addition of glucose. (Figure 9) In case of the two photoautotrophs there was no treatment with glucose addition because no difference was expected due to their inability of ingesting bacteria.
Curves were fitted using thin plate regression splines (aka generalized additive models; Wood et al. 2003).

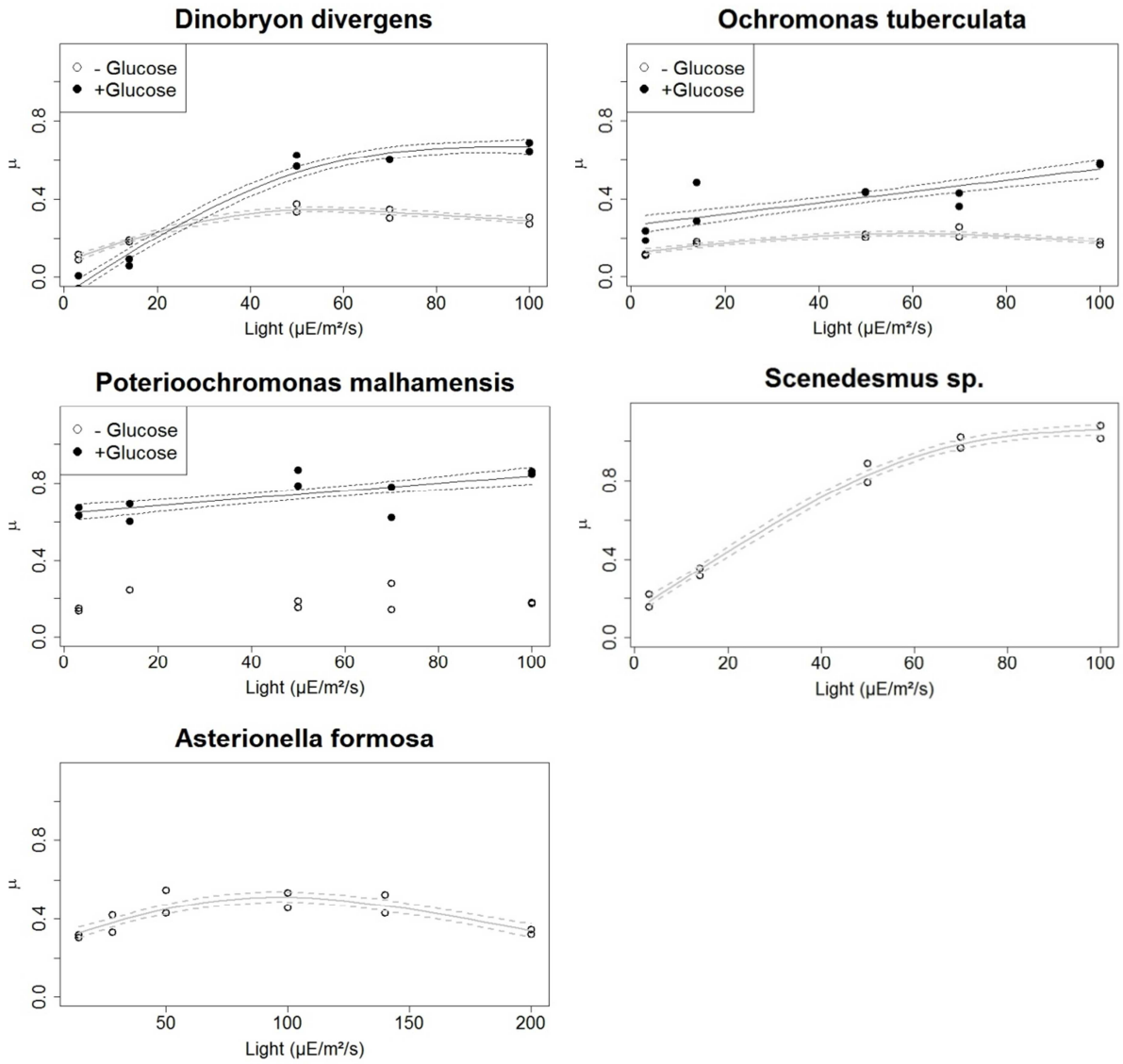



**Figure 9** Growth rates per day of the three mixotrophs *Dinobryon divergens*, *Ochromonas tuberculata* and *Poterioochromonas malhamensis* and of the two phototrophs *Asterionella formosa* and *Scenedesmus sp.* The cultures were kept under different light levels and in a second treatment mixotrophs were also grown with glucose to stimulate bacterial growth (indicated by black dots).

The green alga *Scenedesmus sp*. and the diatom *A. formosa* reached light saturation at around 80μEm$^{-2}$s$^{-1}$ and 50μEm$^{-2}$s$^{-1}$. For the two mixotrophic species *D. divergens* and *O. tuberculata* saturating light intensity was about 50μEm$^{-2}$s$^{-1}$. *Asterionella* indicated decreasing growth rates at light intensities above 100μEm$^{-2}$s$^{-1}$. However, growth rate was higher at low light intensities, compared to the other species. For *Asterionella* it was still about 0.3 day$^{-1}$ at only 14μEm$^{-2}$s$^{-1}$.

Growth rates of *Asterionella formosa* were surprisingly low. In other experiments even at only 20μEm$^{-2}$s$^{-1}$ growth rates of 0.6 day$^{-1}$ were observed. (Holm, 2003) A reason might be that cultures were still contaminated with *Scenedesmus sp*. and *Ankyra sp*. after isolation from Lake Lunz. Although at first contaminating cells were not even detectable and could not be found before at least one week of cultivation, it might have had an influence on the diatom.

Glucose addition resulted in an increase of growth rates in all three mixotrophs. The effect was strongest in *P. malhamensis*. *D. divergens* still reached light saturation at the same light level as without glucose, while *O. tuberculata* and *P. malhamensis* did not reach light saturation when glucose was added.

Growth rates of *P. malhamensis* without glucose addition did not change under different light intensities. When glucose was added, overall growth rates increased and light levels had a slight effect on algal growth.

Comparison of mixotrophic and photoautotrophic growth rates

The results of my experiments reveal a tendency that chrysophytes have got lower maximum growth rates than the green alga *Scenedesmus*. Therefore, I wanted to statistically test the growth rates of more mixotrophic and photoautotrophic species to see if this might be a general pattern.

Being generalistsis, mixotrophs are expected to have lower maximum growth rates than photoautotrophic species due to the combination of both phototrophic and heterotrophic cell structures. (Raven 1997)

To test this assumption, data was taken from the literature to compare maximum growth rates of these two strategies. (Table 1) Data was chosen based on the conditions that algae were grown in monoculture and the treatment was not nutrient limited. To these data I included the results of my own experiments. Sample size for statistical analysis was 20, eight mixotrophic and twelve photoautotrophic organisms.

**Table 1** Species chosen for comparison of mixotrophic and photoautotrophic growth rates. Beside species names and maximum growth rates per day (µmax), also the family, habitat and several parameters under which algae were grown, are given. Regarding nutritional strategy, p stands for phototrophic and m indicates mixotrophy.

| species | family | habitat | light (µEm-2s-1) | light cycle | temperature | µmax | nutritional strategy | author | year |
|---|---|---|---|---|---|---|---|---|---|
| Skeletonema_costatum | Bacillariophyceae | marine | 150 | 16 | 15 | 2,08 | p | Burkhardt | 1999 |
| Thalassiosira_punctigera | Bacillariophyceae | marine | 150 | 24 | 15 | 0,929 | p | Burkhardt | 1999 |
| Asterionella_glacialis | Bacillariophyceae | marine | 150 | 16 | 15 | 1,831 | p | Burkhardt | 1999 |
| Coscinodiscus_wailesii | Bacillariophyceae | marine | 150 | 16 | 15 | 0,579 | p | Burkhardt | 1999 |
| Dunaliella_tertiolecta | Chlorophyceae | marine | | 24 | 19 | 1,113 | p | Godmann | 1979 |
| Gymnodinium_galatheanum | Dinophyceae | marine | | 18 | 15 | 0,3002 | m | Nielsen | 1996 |
| Oscillatoria_agardhii | Cyanobacteria | freshwater | 37 | 24 | 15,4 | 0,501 | p | Ahlgren | 1985 |
| Scrippsiella_trochoidea | Dinophyceae | marine | 150 | 24 | 15 | 0,605 | m | Burkhardt | 1999 |
| Synechococcus_linearis | Cyanobacteria | freshwater | | 24 | | 1,426088 | p | Healey | 1985 |
| Cyclotella_meneghiniana | Bacillariophyceae | freshwater | 210 | 16 | 25 | 0,95 | p | Shafik | 1997 |
| Trichodesmium | Cyanobacteria | marine | 150 | 14 | 25 | 0,5 | p | Ramos | 2007 |
| Phaeodactylum_tricornutum | Bacillariophyceae | marine | 150 | 24 | 15 | 1,515 | p | Burkhardt | 1999 |
| Dinobryon cylindricum | Chrysophyceae | freshwater | 150 | 24 | | 0,46 | m | Caron | 1993 |
| Karlodinium veneficum | Dinophyceae | marine | 120 | 12 | 18 | 0,53 | m | Calbet | 2011 |
| Ochromonas minima | Chrysophyceae | marine | 60 | 16 | 16 | 0,6 | m | Floeder | 2006 |
| Fragilidium subglobosum | Dinophyceae | marine | 160 | 16 | 15 | 0,47 | m | Skovgaard | 1996 |
| Ochromonas tuberculata | Chrysophyceae | freshwater | 45 | 16 | 20 | 0,64 | m | own data | |
| Dinobryon divergens | Chrysophyceae | freshwater | 90 | 16 | 20 | 0,69 | m | own data | |
| Asterionella formosa | Bacillariophyceae | freshwater | 14 | 16 | 20 | 0,529 | p | own data | |
| Scenedesmus sp. | Chlorophyceae | freshwater | 100 | 16 | 20 | 1,079 | p | own data | |

Of the maximum growth rates shown in Table 1 a boxplot was created, separating values into two groups (m for mixotrophs and p for photoautotrophs) (Figure 10). Maximum growth rates of mixotrophs ranged from 0.3 to 0.7 $day^{-1}$. Those of photoautotrophs were higher, reaching values from 0.5 to 2.08 $day^{-1}$. Phototrophs exhibit larger variability in growth rates than mixotrophic organisms.

Prior to statistical analysis, data were tested for normality and homogeneity of variances by Shapiro-Wilk and Levene Tests. Data had to be log transformed to use the t-test. Test statistics are shown in Table 2. In spite of the moderate sample size (n=20), the comparison reveals a significant difference between the two groups.

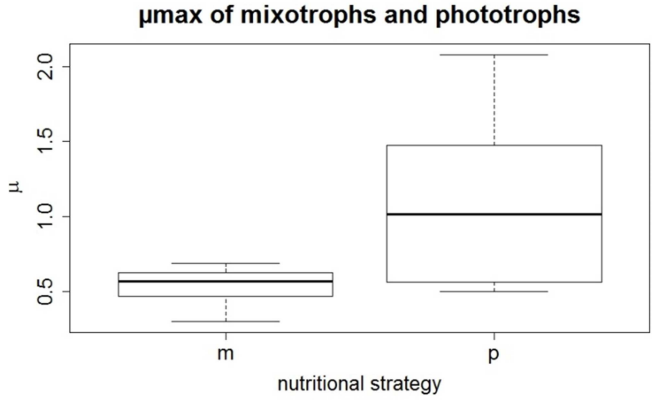



**Figure 10** Comparison of maximum growth rates (µ) of mixotrophic (m) and photoautotrophic (p) phytoplankton.

**Table 2** Results of the t-test to test for differences in maximum growth rates between mixotrophs and photoautotrophs

| | |
|---|---|
| **T test** | -3.1552 |
| **Df** | 18 |
| **p-value** | 0.005476 |
| **Observations** | 20 |
| **Mean (mixotrophs)** | 0,5369 |
| **Mean (photoautotrophs)** | 1,086007 |

Stoichiometric characterization of chrysophytes

Filters for analysis of chlorophyll-a, particulate organic carbon (POC), particulate organic phosphorus (POP) and particulate organic nitrogen (PON) were taken during the exponential and stationary phase of growth. Based on these measurements, ratios of C:P, C:N (molar ratios), chlorophyll-a:C (µg $\mu g^{-1}$) and chlorophyll-a:biovolume (µg $cm^{-3}$) were calculated. (Figures 11 and 12) Species-specific biovolume data was derived from microscopic analysis.

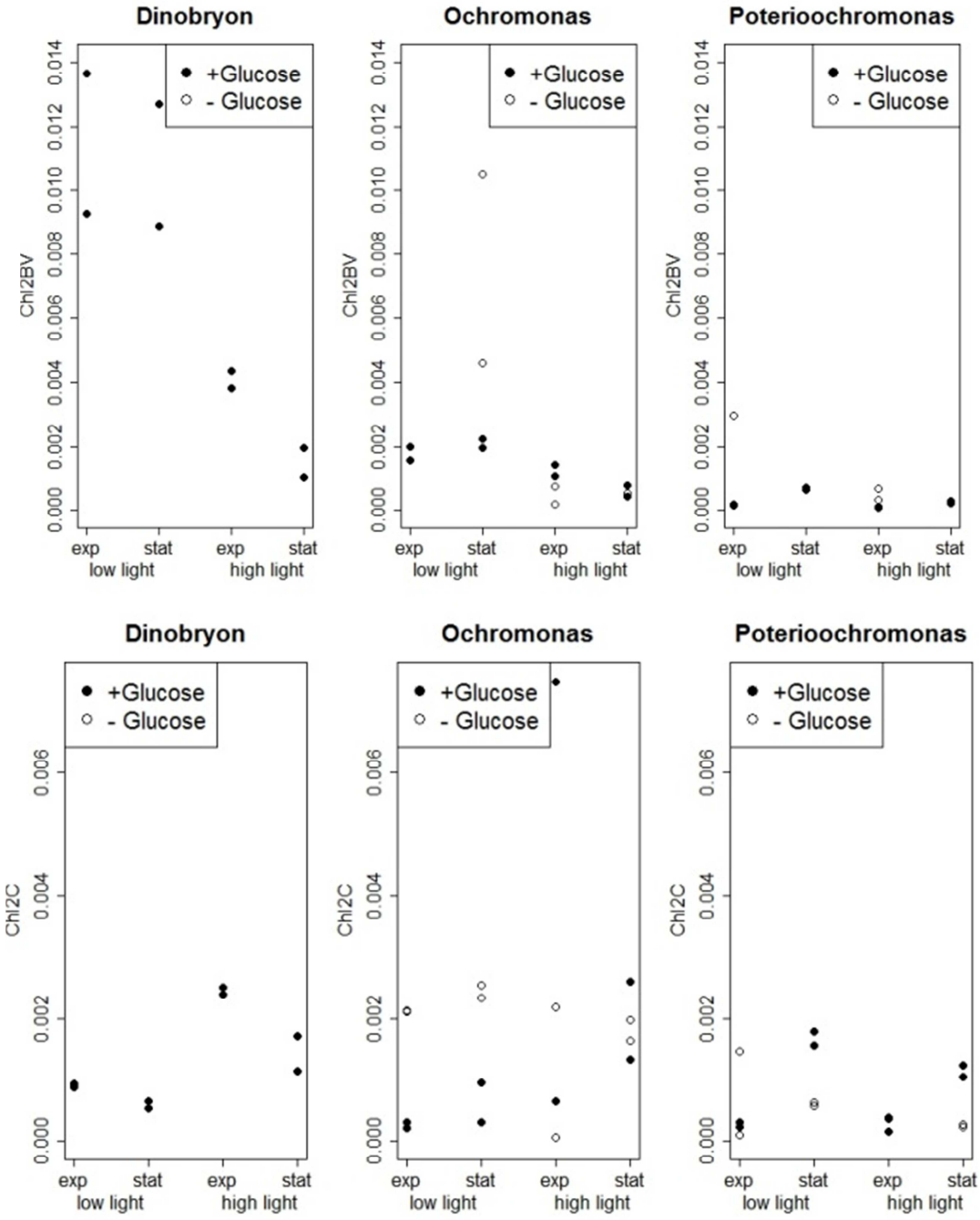



**Figure 11** Ratios of chlorophyll-a:biovolume (µg $cm^{-3}$) and chlorophyll-a:C (µg $µg^{-1}$) of the three chrysophytes *D. divergens*, *O. tuberculata* and *P. malhamensis.*

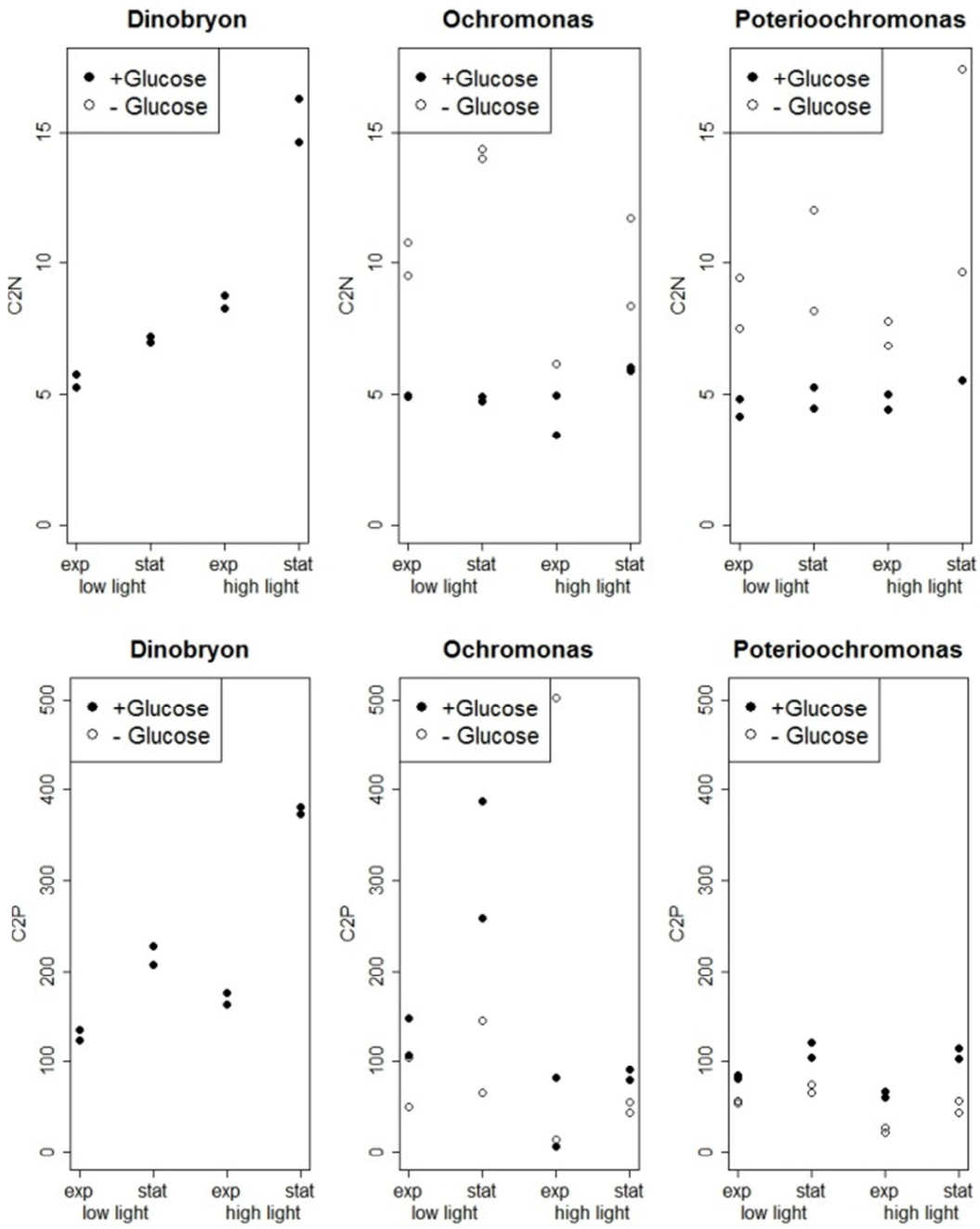



**Figure 12** Ratios of C:N and C:P (molar ratios) of the three chrysophytes *D. divergens*, *O. tuberculata* and *P. malhamensis.*

Unfortunately, the samples of *Dinobryon* cultures without glucose addition could not be measured in time, so these data are missing.

Overall, values of chl-a:C ratios ranged from 0.00005 to 0.007.

In *Dinobryon* cultures chl-a:C was higher under high light conditions. The same pattern was found in *Ochromonas* culture, although the effect was weaker. *Poterioochromonas* revealed the opposite effect, having slightly lower chl-a:C ratios under high light conditions.

Chl-a:C ratio in *Dinobryon* cultures was lower in the stationary phase of growth. In contrast, *Ochromonas* and *Poterioochromonas* showed higher ratios in the stationary phase when glucose was added. No clear pattern was found in the two species without glucose addition.

Chlorophyll-a:biovolume ratios were lowest in *Poterioochromonas malhamensis* cultures, ranging from 0.00007 to 0.003 µg $cm^{-3}$. Highest values were reached by *Dinobryon divergens* cultures at low light intensity (0.0088-0.01 µg $cm^{-3}$). Ratios were especially sensitive to light intensity. In all experiments they tended to be higher when light was limiting.
Similar to the chl-a:C ratio, the highest variation in the chl-a:biovolume data was seen in *Dinobryon* cultures, ranging from 0.001 to 0.01 µg $µg^{-1}$, and lowest in *Poterioochromonas*, ranging from 0.00007 to 0.003 µg $µg^{-3}$.
Chlorophyll-a:biovolume ratios were lower during the stationary phase in *Dinobryon* cultures and in *Ochromonas* cultures at high light intensity with glucose addition. *Ochromonas* at low light levels and *Poterioochromonas* revealed higher ratios in the stationary phase when glucose was added.

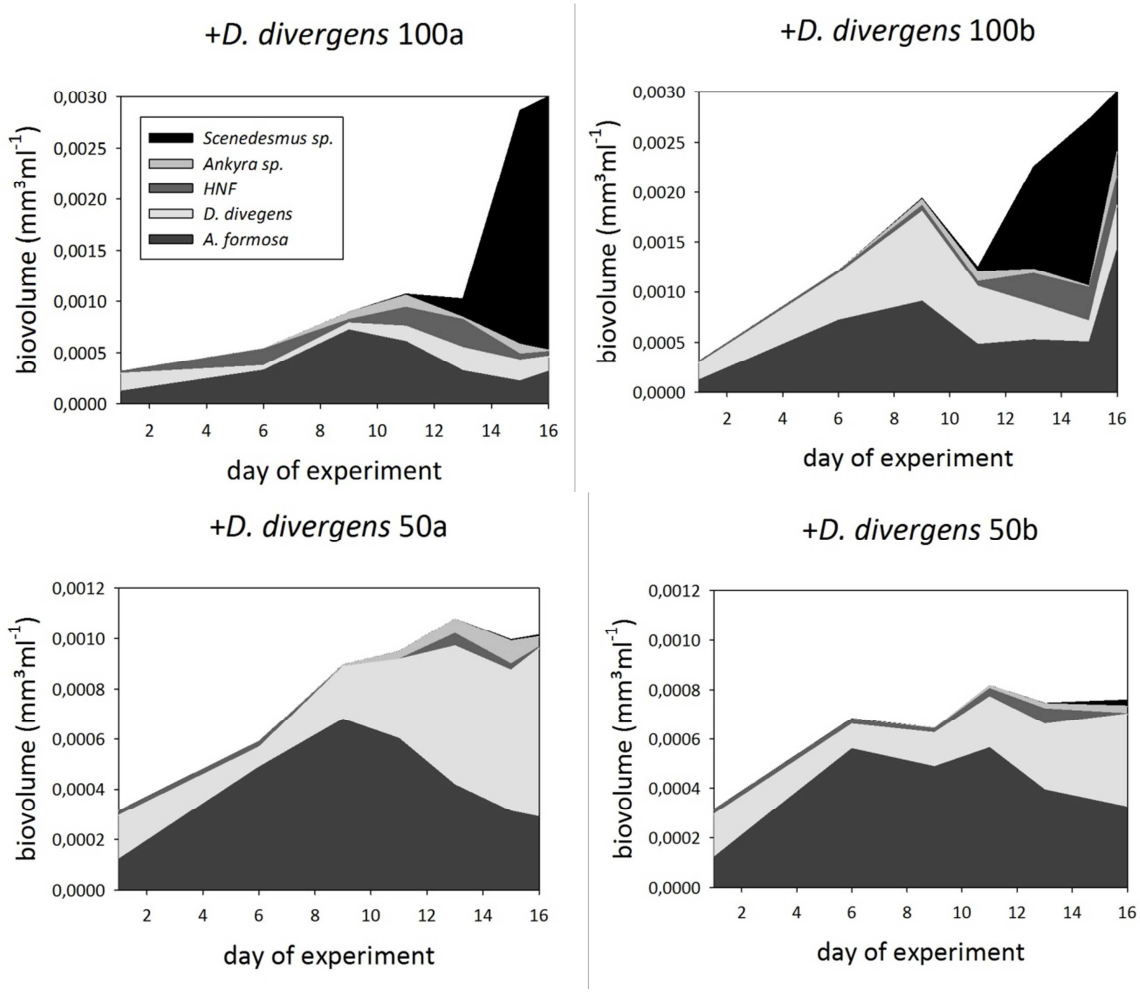
Overall, molar C:N ratios ranged from 3.4 to 17.4. Again, highest values were reached by *Dinobryon divergens* cultures. In *Ochromonas* and *Poterioochromonas* C:N ratios were around 5. In *Dinobryon* cultures C:N ratios were very sensitive to light, revealing higher values at high light intensity. For the other two species, ratios were much more stable.
Molar C:P ratios in *Ochromonas* and *Dinobryon* experiments ranged from around 100 to 400, while *Poterioochromonas* cultures revealed more stable values, around 100. In *Dinobryon* cultures C:P ratios increased at higher light levels, while *Ochromonas* and *Poterioochromonas* reacted in the opposite way, showing lower ratios at high light.
In all cultures and treatments C:P and C:N ratios were higher in the stationary phase of growth than during exponential growth.
Without glucose addition *Poterioochromonas* and *Ochromonas* revealed lower C:P and higher C:N ratios compared to cultures where glucose was added.

It is important to keep in mind that the media used for the cultures were different. For *Ochromonas* and *Poterioochromonas* full WC medium was used, while *Dinobryon* was grown on $SW_{10}$. N:P ratio of full WC medium is 20, so phosphorus is the limiting nutrient, at least to a small extent. Dilution of the medium could have caused differences in nutrient ratios because lake water is clearly P limited. Therefore, the effect of P-limitation is stronger in $SW_{10}$ than in WC medium.

### 3.2 Community Experiment

In the second set of experiments, an artificial community consisting of the diatom *Asterionella formosa*, the mixotroph *Dinobryon divergens* and the heterotrophic nanoflagellate (HNF) *Spumella sp*. was exposed to a threefold light gradient. In order to assess the effects of the mixotroph on the bacteria, there was a treatment without *Dinobryon* in addition to the full community. The experimental communities were grown under constant dilution (exponentially fed batch) to allow for a quasi-steady-state.

*Asterionella formosa* has been isolated from Lake Lunz shortly before starting the experiment. Unfortunately, the two green algae *Scenedesmus sp*. and *Ankyra sp*. were still present in these cultures in very small concentrations. This circumstance led to an invasion of the established communities towards the end of the experiment. However, it did not have a negative influence on the experiment and results still could be analysed.



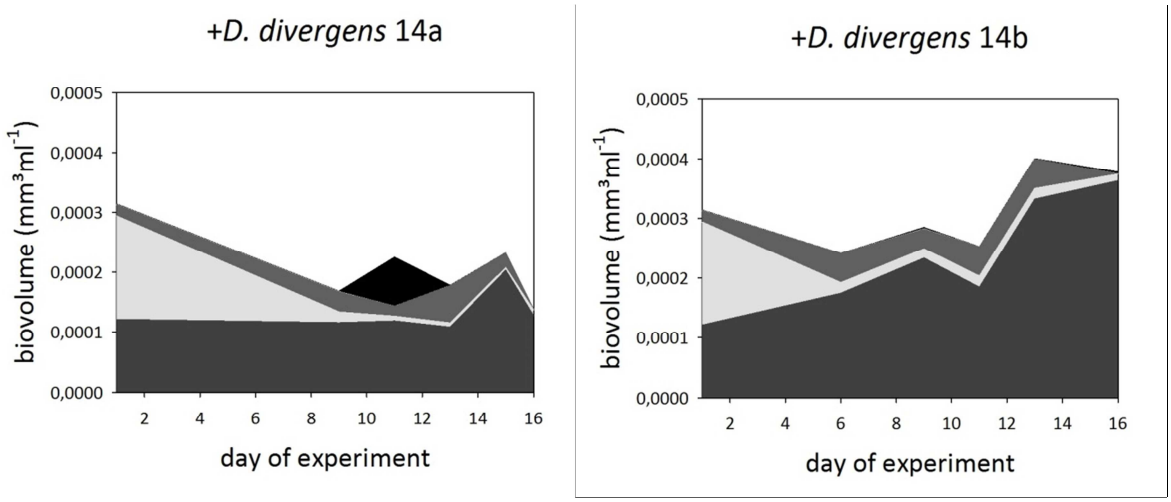



**Figure 13** Growth of the five species *Asterionella formosa*, *Dinobryon divergens*, *Scenedesmus sp*., *Ankyra sp*. and the heterotrophic nanoflagellate (HNF) *Spumella sp*. Biovolume of each organism is plotted against time in days. Due to very different biovolume in the treatments, scaling of y-axes is variable. Also note that at 100µEm$^{-2}$s$^{-1}$ values on day 16 are exceeding the scale.

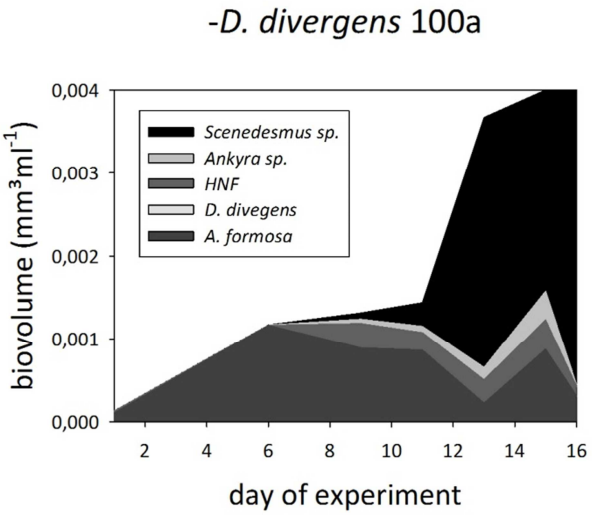
Due to technical problems, the replicates "+Dinobryon 100a" and "+Dinobryon 100b" were treated slightly different. The tube to transport medium did not work for "+Dinobryon 100a", so I had to exchange the medium manually every day. Therefore, in this replicate, medium was not added continually, which can result in different development of the replicates. By the end of the experiment overall biomass in replicate b, with continuous medium dilution, was higher than in replicate a (Figure 13).

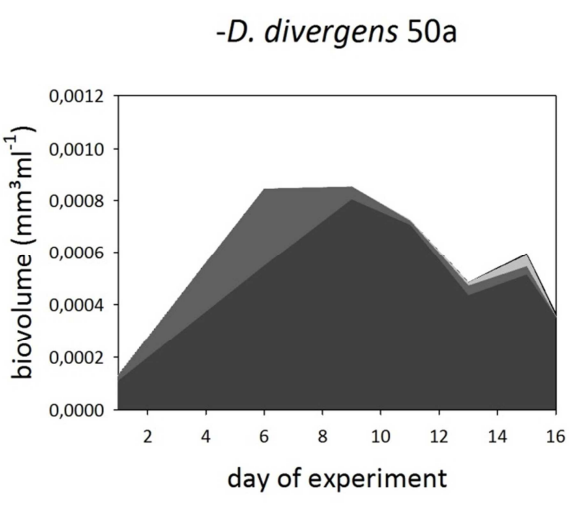
Overall biovolume increased at 100 and 50µEm$^{-2}$s$^{-1}$. The green alga was very rare at 50µEm$^{-2}$s$^{-1}$ so in this treatment *Asterionella* and *Dinobryon* could clearly coexist. When light intensity was higher (100µEm$^{-2}$s$^{-1}$), these two species first showed an increase in biovolume. From day 9, biovolume of *Asterionella* and *Dinobryon* decreased and *Scenedesmus* started invading the culture on day 11. On the last day of the experiment (day 16), it accounted for most of the total biomass.

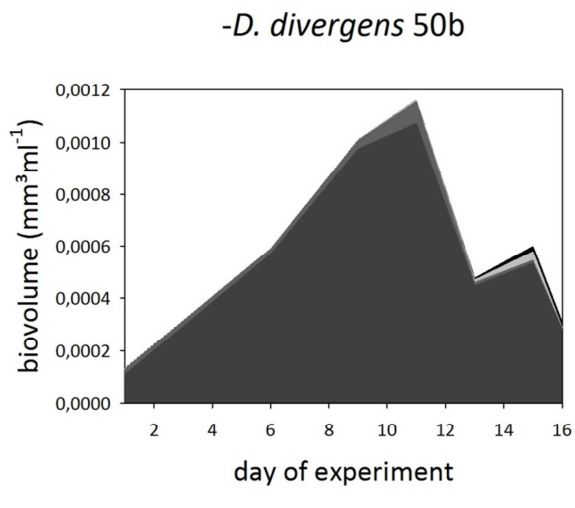
The situation in the communities exposed to low light (14µEm$^{-2}$s$^{-1}$) was quite different. During the first days of the experiment overall biovolume decreased and started to increase again around day 9. As in the treatment of intermediate light intensity, *Scenedesmus sp.* was not able to invade. In this case the amount of light was also too low for the development of the mixotroph. The population decreased during the first days of the experiment. It was present throughout the experiment but at a very low density. In contrast, *Asterionella* was the species that could develop best when light was limiting. It did not show any decrease of biomass, not even at the start of the experiment.

The heterotrophic nanoflagellate was present in all culture flasks throughout the experiment but it did not account for a lot of overall biomass. It had less influence in the communities exposed to 100 or 50µEm$^{-2}$s$^{-1}$ than those at 14µEm$^{-2}$s$^{-1}$, compared to *Dinobryon divergens*. On the last day of the experiment at 14µEm$^{-2}$s$^{-1}$, abundance of HNF showed a sudden and strong decrease but did not disappear completely. However, counting the nanoflagellates sometimes was difficult, which can also cause fluctuations in the data.

The green alga *Ankyra sp.* was present in all culture flasks but it accounted only for a small fraction of total biovolume at all light levels and was not able to become established in the communities.



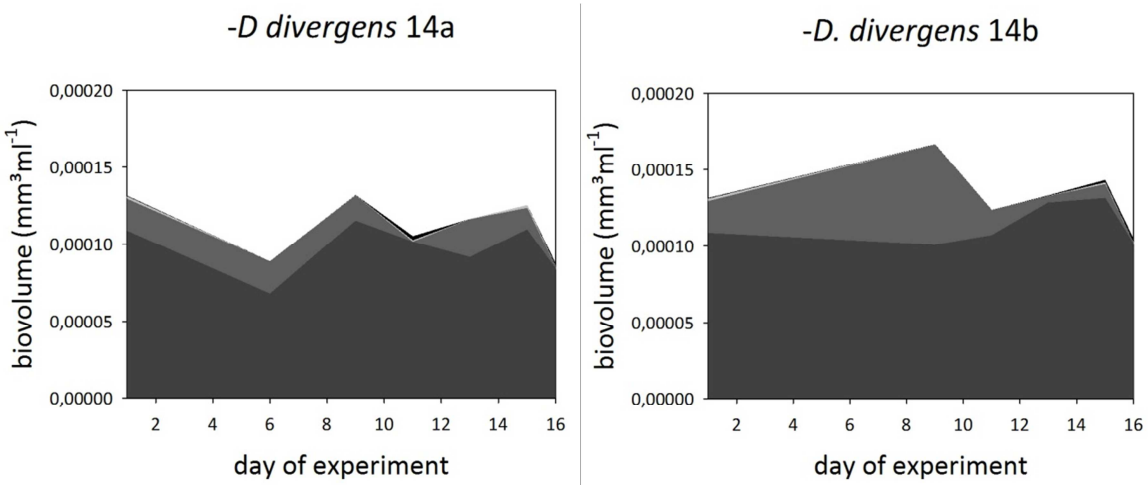
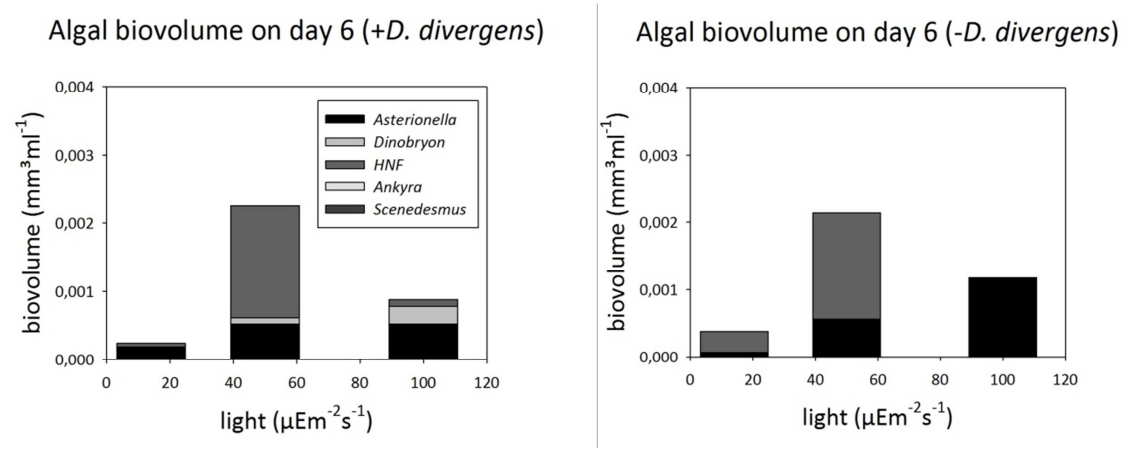



**Figure 14** Same as Figure 13, but for the treatments without *Dinobryon*. Biovolume of each organism is plotted against time in days. Due to very different biovolume in the treatments, scaling of y-axes is variable. Also note that at 100µEm$^{-2}$s$^{-1}$ values of days 15 and 16 are exceeding the scale.

In treatments without the mixotroph, *Scenedesmus sp*. became very abundant at 100µEm$^{-2}$s$^{-1}$ from day 11, like in the communities with *Dinobryon divergens* (Figures 13 and 14). At the intermediate and low light, *Scenedesmus* was present in very low concentrations. At 50µEm$^{-2}$s$^{-1}$ *Asterionella formosa* populations gained biovolume rapidly but showed a decrease from day 9 and 11, respectively. At the lowest light level communities even revealed a slight overall decrease of biovolume compared to the start of the experiment.



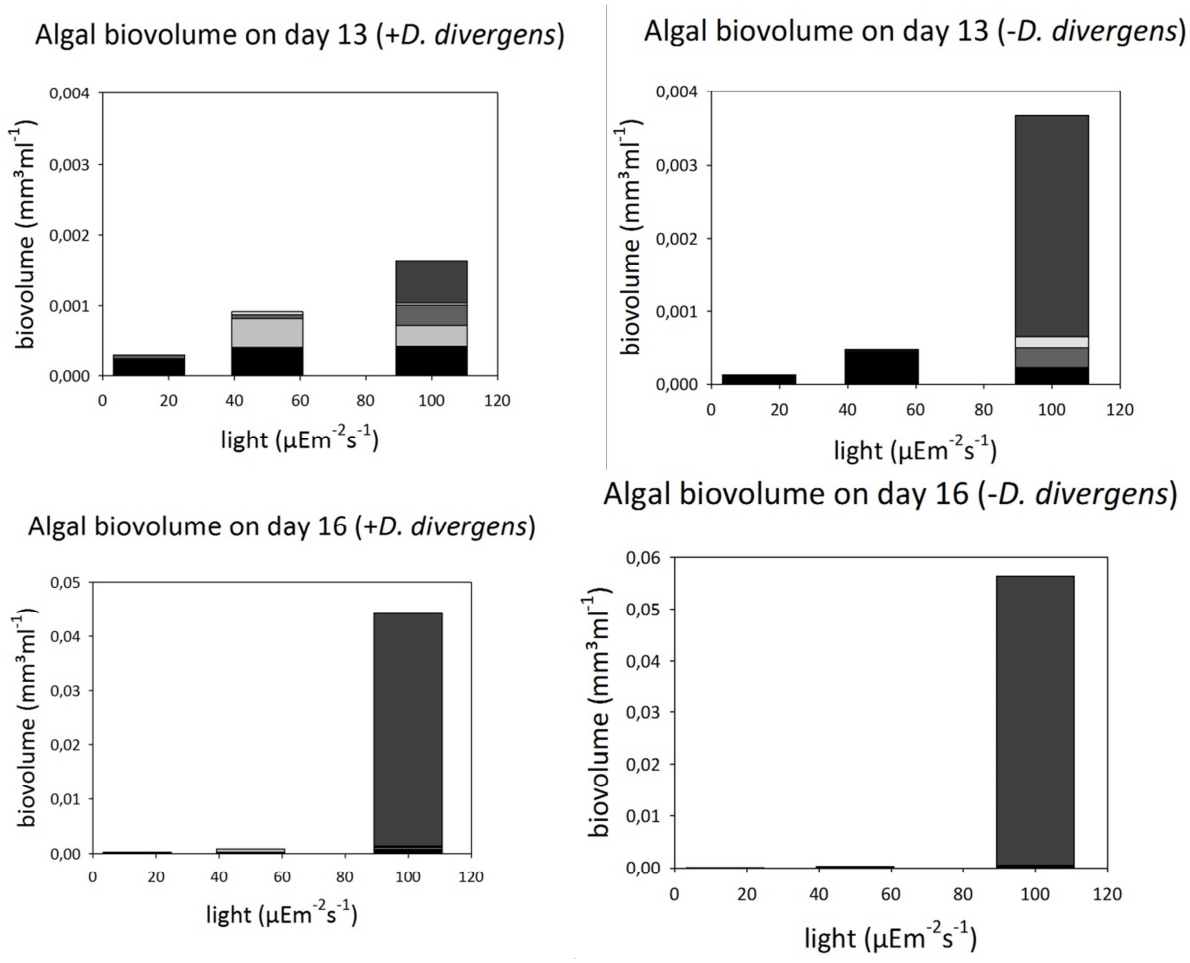



**Figure 15** Development of overall biovolume in the light gradient over time.

Communities at high light levels were much more productive and contained more biovolume towards the end of the experiment than communities at low and intermediate light levels. (Figure 15) However, on day 16 the bulk of biovolume consisted of only one species, the green alga *Scenedesmus sp.*

Relative importance of mixotrophic organisms

The abundance of *Dinobryon* biovolume is important for interpretation of bacteria abundance. To determine relative importance of *Dinobryon* in the different treatments, biovolume of the mixotrophic alga was divided by the sum of mixotrophic plus heterotrophic biovolume (HNF + *Dinobryon*). The results were plotted against light intensity. (Figure 16)

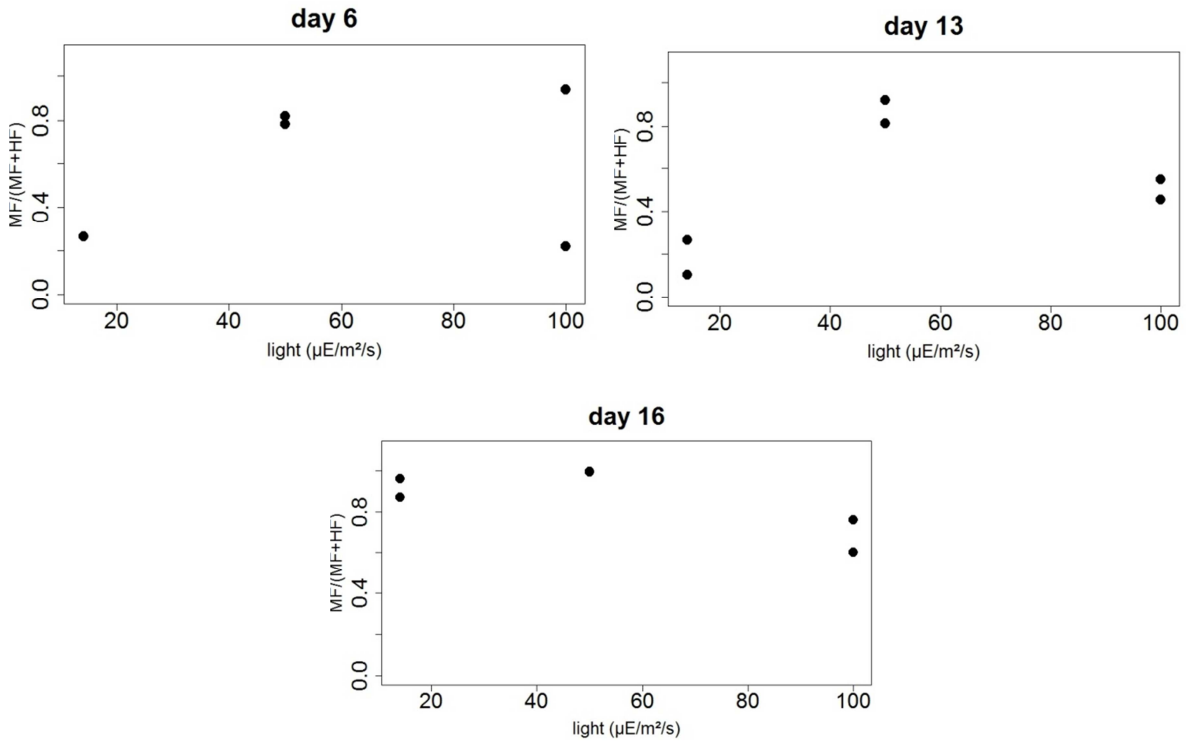



**Figure 16** Relative abundance of *Dinobryon* compared to total bacterivores, plotted against light intensity.

Biovolume of the heterotrophic nanoflagellate at 50µEm$^{-2}$s$^{-1}$ on day 6 had to be corrected because the original value was an outlier. The ratios at 100µEm$^{-2}$s$^{-1}$ on the same day differ a lot from each other. This difference might be generated by the different type of treatment of the culture flasks. Replicate b received a continuous inflow of medium while medium was added manually to replicate a every day. In replicate b the mixotrophic alga obviously had more influence than in replicate a.

Values on the three days revealed a similar pattern. On days 1 and 16 the ratio MF/(MF+HF) is greatest at 50µEm$^{-2}$s$^{-1}$, while ratios at high and low light levels are smaller than at intermediate light. If on day 6 at 100µEm-2s-1 only replicate b is taken into account, influence of the mixotroph increased with increasing light intensity.

On day 16 in the low light treatments MF/(MF+HF) ratios were much higher than on the other two days. This is probably a result of a rapid decrease of HNF abundance.

Bacteria abundance

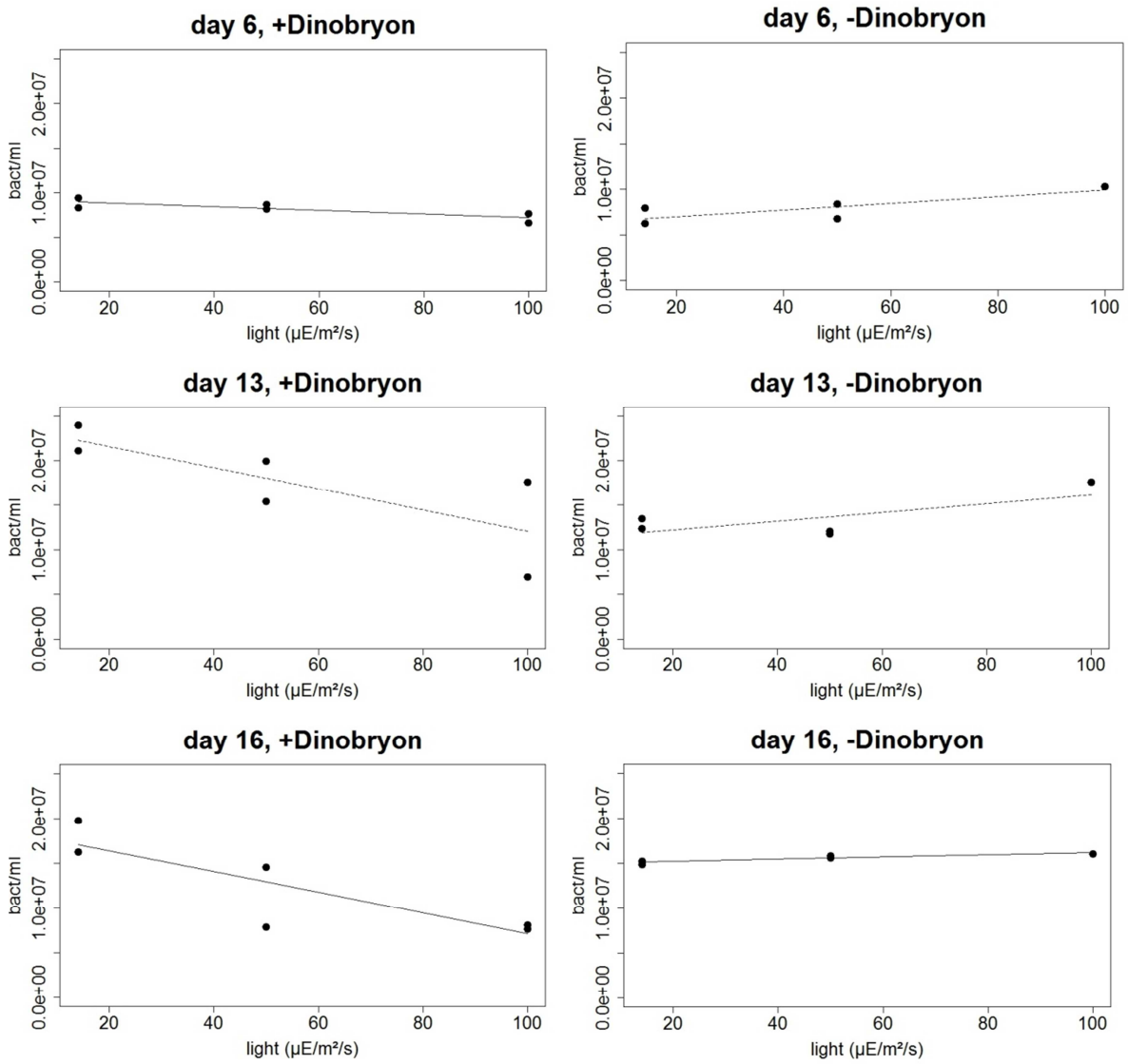



**Figure 17** Bacteria abundance in dependence on light intensity in the established communities with (left column) and without (richt column) *Dinobryon divergens*. The plots show distribution of bacteria on day 6, 13 and 16. Regression lines were fitted to the figures. Solid lines indicate significant results, dashed lines are not significant.

Communities containing mixotrophic algae reveal similar patterns. At each time point bacteria abundance generally decreased with light intensity. In contrast, for communities without mixotrophs the opposite situation was observed: here bacteria abundance increased with light intensity. This effect was slightly stronger on days 6 and 13 than on day 16. (Figure 17)

Test statistics of regression lines are summarized in Table 3. In both treatments on day 13 and in the community without *Dinobryon* on day 6, trend lines were not significant. They still show the tendency of bacteria distribution along the light gradient.

**Table 3** Test statistics of bacteria abundance in the samples taken on days 6, 13 and 16.

| | F statistics | p-value | R squared | Observations |
|---|---|---|---|---|
| **Day 6, + Dinobryon** | 9.748 | 0.03545 | 0.6363 | 6 |
| **Day 13, + Dinobryon** | 5.894 | 0.07216 | 0.4946 | 6 |
| **Day 16, + Dinobryon** | 10.58 | 0.0313 | 0.6571 | 6 |
| **Day 6, - Dinobryon** | 5.353 | 0.1037 | 0.5211 | 5 |
| **Day 13, - Dinobryon** | 3.212 | 0.171 | 0.3561 | 5 |
| **Day 16, - Dinobryon** | 12.87 | 0.03707 | 0.748 | 5 |

A multiple linear regression model was tested to find out whether light levels or presence of *Dinobryon* or the interaction of both factors had an effect on bacteria abundance. (see figure 18) Results of the test statistics are provided in Tables 4-6.

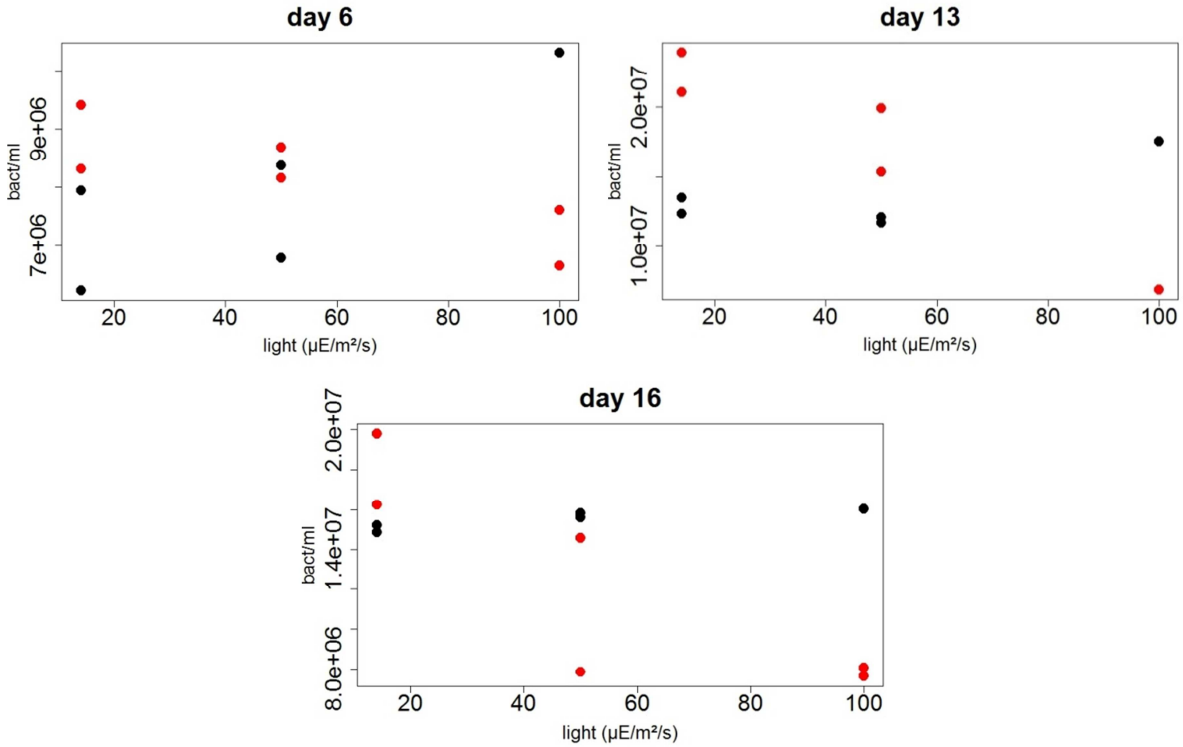



**Figure 18** Bacteria abundance on day 6, 13 and 16 plotted in dependence on light intensity and presence or absence of *Dinobryon divergens*. Black dots indicate communities without *Dinobryon*, red dots indicate those with the mixotroph

**Table 4** Results of the test statistics on day 6.The influence of the two factors light and presence/absence of mixotroph were tested statistically. A multiple linear regression model was created including the two predictors.

| Day 6 | t-value | p-value | Observations |
|---|---|---|---|
| **light** | 3,036 | 0,01896 | 11 |
| **Mixotroph** | 3,262 | 0,01383 | 11 |
| **Interaction** | -3,689 | 0,00777 | 11 |

| F statistics | p-value | R squared | Observations | Predictors |
|---|---|---|---|---|
| **4,62** | 0,04377 | 0,5206 | 11 | 2 |

**Table 5** Results of the test statistics on day 13 (same parameters as in table 4)

| Day 13 | t-value | p-value | Observations |
|---|---|---|---|
| **light** | 1,000 | 0,35069 | 11 |
| **Mixotroph** | 3,407 | 0,01134 | 11 |
| **Interaction** | -2,667 | 0,03214 | 11 |

| F statistics | p-value | R squared | Observations | Predictors |
|---|---|---|---|---|
| **4,555** | 0,04517 | 0,5161 | 11 | 2 |

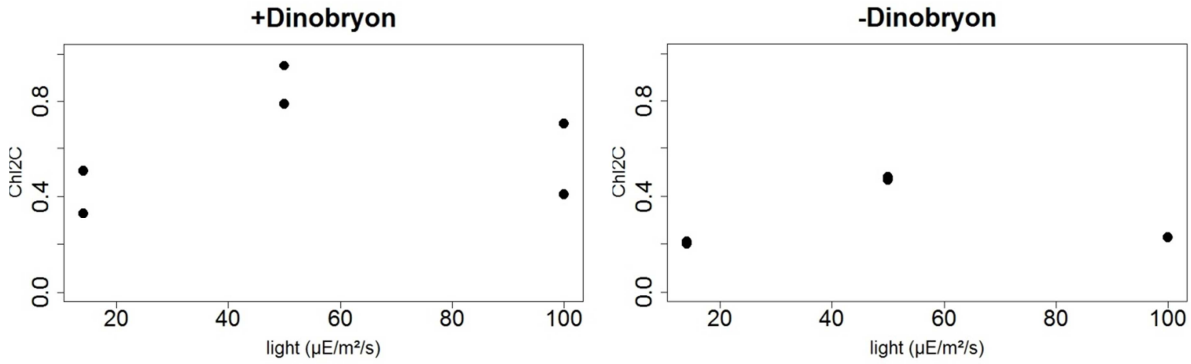
**Table 6** Results of the test statistics on day 16 (same parameters as in table 4)

| Day 16 | t-value | p-value | Observations |
|---|---|---|---|
| **light** | 0.369 | 0.7231 | 11 |
| **Mixotroph** | 1.469 | 0.1854 | 11 |
| **Interaction** | -3.004 | 0.0198 | 11 |

| F statistics | p-value | R squared | Observations | Predictors |
|---|---|---|---|---|
| **7.864** | 0.01209 | 0.6731 | 11 | 2 |

According to the results, light and presence of mixotrophs cause an interactive effect on bacteria abundances on all three sampling days. The influence of mixotrophs alone is significant on days 6 and 13 and the influence of light alone is only significant on day 6.

Seston stoichiometry and chlorophyll-a content



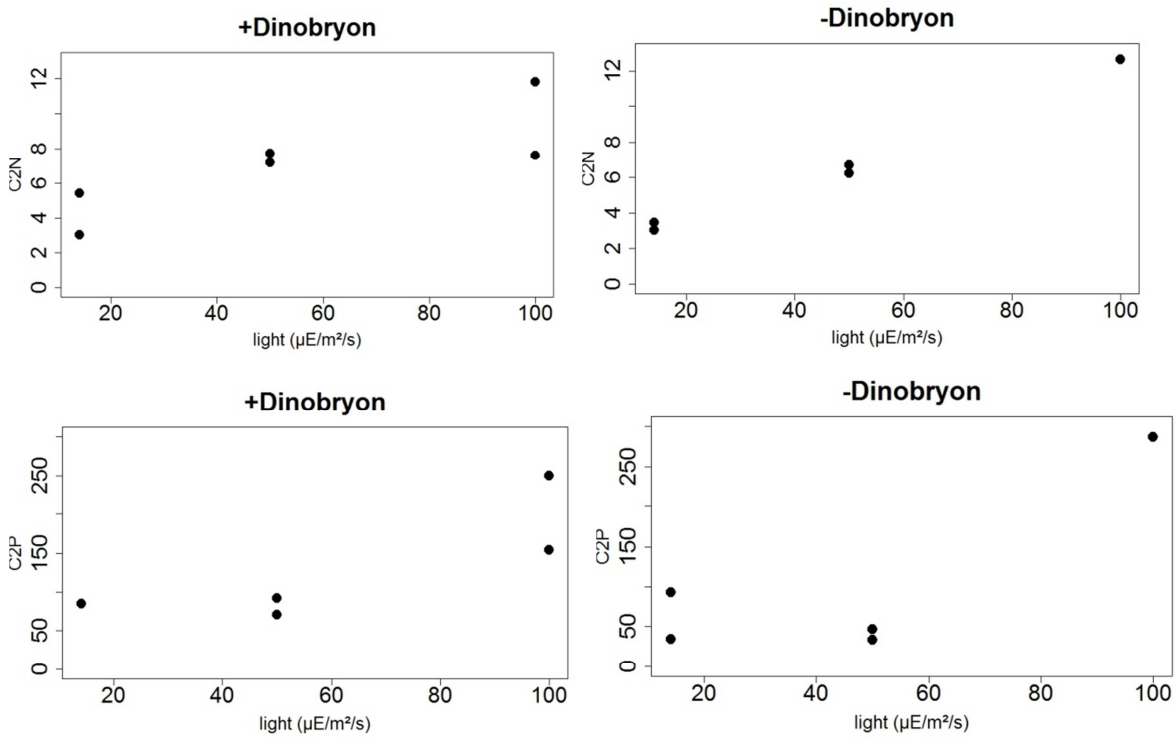



**Figure 19** Chl:C (µg $cm^{-3}$) and molar C:N and C:P ratios in dependence on light intensity in the food web experiment. The left column shows the treatments with, the right column those without *Dinobryon*. Data were collected on day 16.

Chl:C ratios were higher in communities with the mixotroph, ranging from 0.3 to 0.9 µg $cm^{-3}$. Values in cultures without *Dinobryon* ranged from 0.2 to nearly 0.5 µg $cm^{-3}$. In both cases cultures contained more chlorophyll-a at intermediate light intensity.

Molar C:N and C:P ratios increased with increasing light intensity. Values were quite similar in the communities, independent on presence or absence of mixotrophic algae. C:N ratios ranged from 3 to 12.6. C:P values were between 33 and 286. (Figure 19)

# 4. Discussion

### 4.1 Differences in growth rates between mixotrophs and photoautotrophs

A compilation of published data revealed differences in growth rates between mixotrophic and photoautotrophic species. (e.g. Raven 1997, Katechakis and Stibor 2006, Jones 2000) Growth rates of mixotrophic algae are lower than those of specialized phototrophic organisms. Statistically testing 20 mixotrophic and photoautotrophic species resulted in a significant effect. On average, growth rates of mixotrophs were 0.5 $day^{-1}$, while photoautotrophs achieved rates of 1.09 $day^{-1}$, which confirms **Hypothesis I**.

It is important to keep in mind that growth of phytoplankton always depends on culture conditions and can vary over different observations. It is therefore rather surprising to get a significant difference from a limited number of observations, which confirms the strength of the effect. Besides, maximum growth rates might have been slightly underestimated in the experiments, as most growth rates estimated for mixotrophic algae are obtained by growing them photoautotrophically. According to my own data, mixotrophs reveal higher growth rates when bacteria are supplied. This aspect should be taken into account in further studies.

### 4.1 Characterization of selected chrysophytes in terms of growth rates, light requirements and cellular composition

Maximum growth rates

Despite their close relation[1], species differ considerably in terms of their physiological traits along the light gradient.

By ingestion of bacteria*, D. divergens* takes up (limiting) nutrients but cannot compensate unmet energy demands. *P. malhamensis* is able to live heterotrophically and prefers this type of nutrition. The third species, *O. tuberculata*, lies in the middle of these two nutritional strategies. (Figure 9)

The results are consistent with data from the literature. Studies suggest that increasing importance of photosynthesis in a species results in decreasing growth rates, due to higher resource requirements for maintenance of the photosynthetic apparatus. (*Poterioochromonas malhamensis* > *Ochromonas sp.* > *Dinobryon sp.*) (Boenigk et al. 2006) In my experiments when glucose was added, maximum growth rates of *Dinobryon* and *Ochromonas* were indeed lower than maximum growth rates of *Poterioochromonas*. (see Figure 9)

Information on different *Dinobryon* species is scarce. The few existing studies revealed different bacteria clearance rates, so probably even members of the same genus are unequally dependent on bacterivory. (Caron et al. 1993, Jones 1997) In laboratory experiments *Dinobryon cylindricum* was not able to grow in axenic cultures in the long term (Caron et al. 1993), nor could *Dinobryon divergens* grow in complete darkness. (Rottberger et al. 2013)

It has been suggested that *Dinobryon divergens* uses phagotrophy as a strategy to compensate a lack of energy when light is limiting. (Jones 1997) However, my results of growth rates of *D. divergens* are more consistent with results of experiments with *Dinobryon cylindricum*. (Caron et al. 1993) In this case, algae are supposed to gain nutrients for growth by ingesting bacteria.

*D. divergens* revealed very low growth rates when exposed to 3 and 14µEm$^{-2}$s$^{-1}$. This finding leads to the conclusion, that the species cannot cover its requirements for energy from ingested bacteria, and

[1] All algae used in the experiement belong tot he group of Ochromonadales within the Chrysophyceae

is in obvious contradiction with the two other chrysophytes, which achieved positive growth at low light when bacterial growth was enhanced by glucose addition . The even lower growth rates of *Dinobryon* in cultures exposed to low light levels plus glucose addition might be a result of competition with bacteria for dissolved nutrients. Due to their more favourable surface:volume ratio, bacteria are most efficient in uptake of dissolved nutrients when concentrations are limiting. (Currie and Kalff 1984)

Not only growth rates along the light gradient show that *Dinobryon divergens* must be more on the photoautotrophic end of the metabolic spectrum. Cellular chlorophyll-a content is high, the effect of photoadaptation is strong and C:nutrient ratios are quite flexible, which are all typical observations in photoautotrophic plankton species.

In a field study, photosynthesis and bacterivory in *Dinobryon sp*. were observed at different depths. At the surface of the lake, photosynthesis by *Dinobryon sp.* was greatest, while at 3m depth it accounted for only a fraction of the carbon taken up. At 4m depth where light intensity was low, ingestion of bacteria was highest. In this study bacterivory seemingly supplemented the energy requirements of *Dinobryon* at low light, which is obviously in conflict with our findings for *D. divergens*. (Bird and Kalff 1987) Unfortunately, we do not know if several different *Dinobryon* species were found along the water column or if it was only one. Either way, I would consider these findings an indication of great flexibility in the *Dinobryon* genus.

According to several studies, *Poterioochromonas malhamensis* grows primarily heterotrophically while phototrophic growth becomes important only at low bacteria densities. (Caron et al. 1990, Rottberger et. al. 2013, Sanders et al. 1990, Rothhaupt 1996 a & b, 1997) This switch to phototrophy is indicated by an increase of chlorophyll-a content. However, phototrophy did not contribute more than 7% to the organism's carbon budget. (Sanders et al. 1990) Furthermore, the rate of photosynthesis varies greatly, depending on the amount of light available, while bacteria ingestion rates proved to be more constant. (Sanders et al. 1990) Growth of *P. malhamensis* is very slow on inorganic medium without bacterial prey. Addition of organic compounds has got no or a very small effect on growth rates. Only when bacterial prey is available growth rates of *P. malhamensis* increase significantly (Sanders et al. 1990, Holen 1999, Pålsson et al. 2004).

My results as well lead to the conclusion that *P. malhamensis* depends strongly on ingestion of bacteria. Without glucose addition, growth rates remained similarly low, irrespective of the light intensity. Growth rates were 3-4 times higher when glucose was added to trigger bacterial growth. (Figure 9) Another study (Holen 1999) found phagotrophic growth rates five times higher than autotrophic growth rates. Again growth rates did not differ much between the light and dark treatment when bacteria were present. Together with the very low chlorophyll-a content in the cells

and the low variability of C:nutrient ratios, these results clearly show that *Poterioochromonas* is located very much on the heterotrophic end of the metabolic spectrum.

Several *Ochromonas* species use photosynthesis in addition to bacterivory when prey is limiting. Studies revealed that heterotrophic growth of an *Ochromonas* strain leads to much higher growth rates compared to mixotrophic growth. (Boechat et al. 2007) The marine species *Ochromonas minima* uses both metabolic pathways simultaneously and is not able to achieve positive growth in the darkness irrespective of prey concentration. (Floeder et al. 2006)
These studies are consistent with my results. Obviously *Ochromonas tuberculata* cannot be classified as mainly phototrophic or phagotrophic. Under low bacteria concentrations growth rates were lower than those of *Dinobryon divergens*. It responded strongly to the addition of glucose but still did not reach growth rates as high as *Poterioochromonas malhamensis*.

Bacterivory in photosynthetic phytoplankton is considered to provide two main resources (Pålsson and Granéli 2003, Caron et al. 1993)

- energy for cell maintenance and growth
- growth factors and nutrients like nitrogen or phosphorus

Thus, it can be a strategy for survival when light is limiting. (Caron et al. 1993) In this study the aim was to find out which of these aspects is more relevant in Chrysophytes and to what extent they are able to use bacteria as a source of energy. As results suggest, in only the three chrysophytes studied, the whole spectrum can be found.

Nygaard and Tobiesen (1993) suggested that the uptake of phosphorus by bacterivory is more important for mixotrophs than the uptake of carbon. Bacterivory rates become higher when P is limiting and are higher in the surface samples than in those from deeper water layers. If algae used bacterivory to gain carbon, the expectation would be an increase of bacterivory with depth and decrease of light.

The three species used in my experiments, indicated higher growth rates when glucose was added to trigger bacterial growth. Information on different mixotrophic species mentioned above leads to the same conclusion, which is consistent with **Hypothesis II** that higher bacterial abundance will lead to higher maximum growth rates in the mixotrophs.

Saturating light levels

Because mixotrophic algae are supposed to use light less efficiently than green algae (Holen and Boraas, 1995), I suggested that chrysophytes reveal higher light saturating levels than photoautotrophic algae (**Hypothesis III**). In the experiments, saturating light levels for the three chrysophytes, one green algae, *Scenedesmus sp*., and a diatom, *Asterionella formosa* were evaluated. Contrary to the expectation, results suggest that chrysophytes have got lower saturating light levels than *Scenedesmus sp*., which is obviously more adapted to higher light intensities. In contrast, *Asterionella formosa* revealed saturating light levels more similar to chrysophytes.

Another study (Talling, 1957) also revealed saturating light levels of around 40µEm$^{-2}$s$^{-1}$ in *Asterionella sp*. In this case photosynthetic rates were estimated instead of growth rates but as these rates should be well correlated, results can be compared.

Although *Dinobryon divergens* and *Asterionella formosa* revealed very similar light saturating levels, one species is abundant in surface waters while the other one is more abundant at greater depth in Lake Lunz. (Figure 2) My results show that this cannot be explained only by light saturation. Beside, also maximum growth rate is an important parameter defining, where in the water column a species has got its preferred habitat.

Stoichiometric characterization of chrysophytes

The chlorophyll-a:C ratio in phytoplankton increases with decreasing light intensity. (e.g. Geider et al. 1996) This phenomenon is known as photoadaptation. Chl-a content is adjusted depending on growth conditions. More chl-a is needed to grow at low light levels. Reasons for a decrease of chl-a:C ratios are dilution effects as a result of higher growth rates. At the same time, under conditions of high light:low nutrients, too much light can damage the cell, as the cell cannot turn the light energy into new biomass. Low chl:C ratio protects a cell from photoinhibition. (Behrenfeld et al. 1998) In such situations, chlorophyll-a is decomposed at a specific rate which increases with light intensity. (Zonneveld 1998) At low light levels, high chl-a:C ratios cause a “package” effect. While the amount of chlorophyll-a is relatively large, the cell volume remains small which leads to a shading effect and to a decreasing efficiency of light capture per unit of chlorophyll-a. (Zonneveld 1998)

Chlorophyll-a:biovolume ratios meet the expectations quite well. Chlorophyll-a content was higher at low light than at high light levels in all three species. It was lower during the exponential phase of growth than during stationary phase, except in *Dinobryon* cultures and in *Ochromonas* cultures exposed to high light levels. In these cultures chlorophyll production must have exceeded the dilution effect. Again, it is also a hint that for these two species light is a more important resource than for *Poterioochromonas*, as they produce larger amounts of chlorophyll-a.

The effect of photoadaptation was strongest in *Dinobryon divergens*. Differences in growth rates were much greater between high light and low light treatments, in line with a higher plasticity in terms of chl-a:C ratio. At the same time, *D. divergens* on average exhibited the highest chl-a:C ratio.
In contrast to the two other species which can grow without light, this species strictly depends on photosynthesis.

Overall, chlorophyll-a content is much lower in *Ochromonas* and *Poterioochromonas* cultures. As these two species can cover their energetic requirements from bacterivory, they are able to maintain lower chlorophyll-a levels, while *Dinobryon* needs to cover energy demands by photosynthesis. For *Poterioochromonas malhamensis* studies revealed a reduction of cellular chlorophyll-a when algae had access to high densities of prey. (Pålsson and Daniel 2004) Contrary to photoautotrophs that reduce chlorophyll-a content to protect the cells in situations of nutrient limitation, mixotrophs like *Poterioochromonas* reduce chlorophyll-a because they prefer a heterotrophic way of nutrition. Thus, they do not need the pigments when prey is available. This can also be an explanation for higher chlorophyll-a content in treatments without glucose addition.
Little information is available about chl-a:biovolume ratios of photoautotrophic algae. In a field study phytoplankton samples from different depths revealed values of 23590 – 530 µg $cm^{-3}$. (Felip and Catalan 2000) Thus, it can be suggested that chlorophyll-a content of the three mixotrophic algae I studied, is much lower than of average phytoplankton communities. This finding is also confirmed when comparing chl-a:C ratios. Values of 0.003 to 0.04 were observed for *Skeletonema costatum* (Sakshaug et al.1988), while chl-a:C ratios in my experiments ranged from 0.00006 to 0.007. However, when interpreting chl-a:C ratios of the three mixotrophs, it is important to keep in mind that measured carbon concentrations include also bacterial carbon. This is especially relevant in samples where glucose was added and bacteria density was high. It is also the reason why I focused on the interpretation of chl-a:biovolume instead of chl-a:C ratios.
According to my data, mixotrophic algae have less chlorophyll-a per unit biovolume than photoautotrophs, as suggested in **Hypothesis IV**.

The stoichiometric composition of algae (C:N:P) is commonly compared to the so-called Redfield-ratio of 106:16:1. This ratio was originally derived from marine seston samples and represents a global average for phytoplankton not limited by light or nutrients (Sterner & Elser 2002). However, this ratio is not a consequence of cell properties but results from a certain balance between N-rich protein and P-rich rRNA (Loladze and Elser 2011). The actual chemical composition of phytoplankton shows great flexibility, depending on species, growth rate, nutrient and light limitation and the species' potential of storing nutrients. (Klausmeier 2008, Hillebrand 2013) According to the light-

nutrient hypothesis postulated by Sterner et al. (1997), C:nutrient ratios depend on the amount of light and nutrients available. Under high light conditions or low nutrient supply phytoplankton tends to reveal high C:P and C:N ratios, which lowers food quality for herbivorous zooplankton.

Because mixotrophs combine photosynthesis with phagotrophy, their cell stoichiometry is more similar to that of heterotrophs and therefore less flexible in terms of its C:N:P ratio, hence less affected by environmental changes compared to photoautotrophs. Due to lower and more stable C:nutrient ratios, mixotrophs have been considered being better food quality for herbivorous zooplankton. (Katechakis, 2005)

Nutrient saturated environments can lead to luxury consumption, i.e. excessive uptake and storage of N or P, and result in lower C:nutrient ratios. (Dickman et al. 2005)
Structural N:P ratios determine nutrient requirements and are species specific. Studies found N:P ratios of 7.1-43.3 depending on the species. (Klausmeier et al. 2004a) The chemical composition of algae is more restricted at high growth rates, whereas it shows broad variation at low growth rates. (Klausmeier et al. 2008, Hillebrand et al. 2013) High growth rates increase cellular phosphorus content due to the increased production of ribosomes that contain large amounts of P. (Klausmeier et al. 2004a, Loladze and Elser 2011)

My results are quite consistent with the light-nutrient hypothesis. All species used in the experiment revealed higher C:N and C:P ratios under high light conditions. The only exception is the C:P ratio in *Ochromonas tuberculata* cultures. Stationary phase of growth can be seen as nutrient limited. As a consequence C:N and C:P ratios were higher during stationary phase of growth in all species and treatments.
However, C:nutrient ratios in *Ochromonas* and especially in *Poterioochromonas* cultures are more stable than those of *Dinobryon divergens*. Overall freshwater phytoplankton biomass reveals C:nutrient ratios much higher and more variable than ratios found in the three mixotrophic cultures. In freshwater habitats C:P ratios of 1000 and more are common and C:N ratios can also reach values of 55 and more. (Sterner and Elser, 2002)
It can be assumed that as *Dinobryon* is more on the phototrophic side of the metabolic spectrum, similar to photoautotrophic algae, it might be more sensitive to changes of light and nutrients than the other two species.

*Ochromonas tuberculata* and *Poterioochromonas malhamensis* cultures show lower C:P ratios in treatments without glucose addition. This result is quite surprising, regarding the importance of

bacterivory for phosphorus uptake in mixotrophic algae, which has often been suggested in the literature. (e.g. Nygaard and Tobiesen 1993) However, C:N ratios were lower in treatments where glucose was added. This may less be a signal from the algae themselves, but an effect of higher detritus production by bacteria in the treatments where glucose was added, due to the availability of glucose. As bacteria were not separated from algae when samples were filtered, this particulate matter was included to nutrient analysis. Detritus and dissolved organic matter may contain a large amount of biologically unavailable nitrogen. (Ptacnik et al. 2010) Conversely, algae and heterotrophic bacteria extract phosphate from dead organic matter using extracellular phosphatases. (štrojsová et al. 2003) Therefore, C-rich particulate matter was P-depleated but still contained large amounts of nitrogen, which led to high C:P but low C:N ratios in the samples with glucose addition.

### 4.2 Community Experiment and implications for natural communities

Species abundance in the communities

In the communities, productivity and therefore overall biovolume increased with increasing light intensity. In treatments including *D. divergens*, the mixotroph was most abundant at intermediate ($50\mu Em^{-2}s^{-1}$) light levels, whereas at low light intensity it accounted for very little of the overall biovolume. In Lake Lunz, peak abundances of *Dinobryon* were also found above those of *Asterionella*, but the shift between the two species occurred at higher light levels (approximately $170\mu Em^{-2}s^{-1}$). According to these results, **Hypothesis V** could clearly be confirmed. While at light levels 100 and 50 $\mu Em^{-2}s^{-1}$ the mixotroph and the diatom were able to coexist, at $14\mu Em^{-2}s^{-1}$ *A. formosa* outcompeted *D. divergens*.

In a natural environment, competition is not only the result of species' growth rate and light saturation levels. Decrease of *D. divergens* populations along the light gradient can have many more reasons not studied during this experiment, e.g. temperature, competition with other species, changing bacteria abundance. Besides, *Asterionella formosa* is supposed to have higher growth rates than observed here. Holm and Armstrong (1981) found growth rates of 0.6, while in my experiments growth rates did not exceed 0.4. This might have lowered the ability of the diatom to compete with *Dinobryon* populations, so the mixotroph revealed better development than expected from field measurements.

Bacteria abundance

Presence of the mixotroph obviously had an effect on bacteria populations, that was also light-dependent. This finding partly confirms **Hypothesis VI**. According to statistical results, there was an interactive effect of presence/absence of *Dinobryon* and light intensity on bacteria abundance.

In communities without the mixotroph, bacteria density increased with increasing light intensity. Grazing pressure is probably lower than in treatments including *Dinobryon divergens*, because the HNF is the only predator ingesting bacteria. With increasing light intensity also *Asterionella formosa* populations became larger in the culture flasks. Those provide an important source of nutrients for bacteria by releasing dissolved organic compounds. (e.g. Mitra et al. 2014)

In communities with the mixotroph bacteria density was especially high at low light intensity. In these treatments *Dinobryon* populations were less abundant but *Asterionella* was the dominant species. Conversely, at high light, bacteria were clearly reduced in treatments with *Dinobryon* compared to those without the mixotroph.

At intermediate light levels Dinobryon populations revealed highest densities, which led to lower bacteria densities, compared to low light levels.

At high light levels, abundance of *Dinobryon* was lower but bacteria populations were still smaller than at intermediate light intensity. An explanation could be that mixotrophs might increase ingestion rates at higher light levels. In a batch experiment, Jones and Rees found an increase in particle clearance rate in treatments where light intensity was low. (Jones 2000) (*Dinobryon divergens* ) On the contrary, Caron et al. (1993) found out that ingestion rates of bacteria were positively correlated with light intensity. (*Dinobryon cylindricum*) The monoculture growth experiments already revealed similarities of *Dinobryon divergens* used in my study to *Dinobryon cylindricum* used by Caron et al. (1993). I would therefore suggest that with increasing light intensity *D. divergens* increases grazing on bacteria to gain more nutrients for increased photosynthesis, which leads to lower bacteria densities.

<u>Seston stoichiometry and chlorophyll content</u>

Overall, C:N and C:P ratios are higher at higher light levels, which is consistent with the light nutrient hypothesis (Sterner et al. 1997) The treatments exposed to 100µEm$^{-2}$s$^{-1}$ revealed highest C:nutrient ratios. Especially in C:P ratios there is a great difference between high light treatments and low or intermediate light treatments. Higher overall productivity leads to an increased production of P-rich ribosomes and an increased P concentration in the cells.

Besides, C:P ratios of photoautotrophic algae are usually higher than those of mixotrophs. As *Scenedesmus sp*. became very abundant at 100µEm$^{-2}$s$^{-1}$, high biomass of this green alga likely explains higher C:P ratios in these treatments.

<u>Ecological implications</u>

My study shows that even closely related chrysophytes differ considerably in their growth kinetics and light demand. In fact, they reveal different modes of nutrition, different growth rates and are

unequally dependent on light and prey abundance. Overall, experiments with mixotrophic species are very scarce. Because mixotrophs are very important as bacterial grazers and many examples are known of mixotrophic algal blooms, further studies on this group of organisms are important for a better understanding of aquatic ecosystems.

Usually mixotrophs are not taken into account in ecosystem modelling and the microbial loop (Azam et. al. 1983), which leads to a misunderstanding of these models. Estimations of primary and secondary production should be corrected considering mixotrophs, as they account for a large fraction of both processes. (Flynn et al. 2012) Traditionally, microzooplankton and especially heterotrophic nanoflagellates were seen as the main contributors of nutrient regeneration. Including mixotrophs into ecosystem models provides another pathway of using inorganic nutrients. By ingesting bacteria, mixotrophs make nutrients available for primary production which cannot be used by phototautotrophic organisms. (Mitra et al. 2014)

Evidence suggests that the amount of mixotrophs in the phytoplankton community plays an important role in terms of food quality for aquatic herbivores. Mixotrophs with their more stable nutrient ratios are supposed to be a better food resource than purely phototrophic algae. They may provide constant food quality for herbivorous zooplankton when light:nutrient ratios change within the ecosystem. (Katechakis et al. 2005)

Chrysophytes are K-strategists and take advantage from ecosystems where either light or nutrients are limiting. This was found out in a laboratory experiment with *Poterioochromonas malhamensis* under food limiting conditions and can be applied on chrysophytes in general. At high food concentrations *P. malhamensis* revealed low growth rates but when prey was limiting also mortality rates were low. (Boenigk et al. 2006)

According to my data, *Dinobryon divergens* should be especially abundant in surface waters, which was confirmed by comparison with algal abundance in Lake Lunz. *Uroglena americana* is another example of a colonial chrysophyte that occurs mainly in surface waters. (e. g. Urabe et al. 1999) Like *Dinobryon*, these colony forming species are obligate phototrophs and have benefits from bacteria ingestion, as additional uptake of phosphorus or other nutrients. *Uroglena* is frequently observed during summer stratification close to the water surface in Lake Lunz.
However, chrysophytes can also occur in deeper water layers when the euphotic zone reaches the hypolimnion. (Bird and Kalff, 1987) In this case they use prey as a source for energy to compensate for limitation of light.

My data shows that within chrysophytes only three species may exhibit a wide variety of nutritional strategies. Although mixotrophs are very abundant and are considered important bacterivores in oligotrophic waters, we still do not know which of those strategies are most widespread within the organisms. Quantitative analyses of phytoplankton in lakes indicate very high abundance of certain species like *Dinobryon* or *Uroglena*, with seasonal variations.

# References


Behrenfeld, M. J., Prasil, O., Kolber, Z. S., Babin, M. and Falkowski, P. G. Compensatory changes in Photosystem II electron turnover rates protect photosyntesis from photoinhibition. Photosynthesis Research 58, 259–268 (1998)

Bird, D. F. & Kalff, J. Algal phagotrophy: regulating factors and importance relative to photosynthesis in Dinobryon(Chrysophyceae). Limnology and Oceanography 32, 277–284 (1987).

Boëchat, I. G., Weithoff, G., Krüger, A., Gücker, B. & Adrian, R. A biochemical explanation for the success of mixotrophy in the flagellate Ochromonas sp. Limnology and oceanography **52,** 1624–1632 (2007).

Boenigk, J., Pfandl, K. & Hansen, P. J. Exploring strategies for nanoflagellates living in a'wet desert'. Aquatic microbial ecology 44, 71–83 (2006).

Brussaard, C. P. D. Optimization of Procedures for Counting Viruses by Flow Cytometry. Applied and Environmental Microbiology 70, 1506–1513 (2004).

Caron, D. A., Porter, K. G. & Sanders, R. W. Carbon, nitrogen, and phosphorus budgets for the mixotrophic phytoflagellate Poterioochromonas malhamensis (Chrysophyceae) during bacterial ingestion. Limnology and oceanography 35, 433–443 (1990).

Caron, D. A., Sanders, R. W., Lim, E. L., Marrasé, C., Amaral, L. A., Whitney, S., Aoki, R. B., Porter, K. G. Light-Dependent Phagotrophy in the Freshwater Mixotrophic Chrysophyte Dinobryon cylindricum. Microbial Ecology 25, 93–111 (1993)

Currie, D. J., Kalff, J. Can Bacteria Outcompete Phytoplankton for Phosphorus? A Chemostat Test. Microbial Ecology 10, 205–216 (1984)

Christensen, U. R. & Tilgner, A. Power requirement of the geodynamo from ohmic losses in numerical and laboratory dynamos. Nature 429, 169–171 (2004).

Dickman, E. M., Vanni, M. J. & Horgan, M. J. Interactive effects of light and nutrients on phytoplankton stoichiometry. Oecologia 149, 676–689 (2006).

Felip, M. and Catalan, J. The relationship between phytoplankton biovolume and chlorophyll in a deep oligotrophic lake: decoupling in their spatial and temporal maxima. Journal of Plankton Research 22, 91–105 (2000)

Fischer, R., Andersen, T., Hillebrand, H. & Ptacnik, R. The exponentially fed batch culture as a reliable alternative to conventional chemostats. Limnology and Oceanography: Methods 12, 432–440 (2014).

Flöder, S., Hansen, T. & Ptacnik, R. Energy–Dependent Bacterivory in Ochromonas minima–A Strategy Promoting the Use of Substitutable Resources and Survival at Insufficient Light Supply. Protist 157, 291–302 (2006).

Flynn, K. J. et al. Misuse of the phytoplankton-zooplankton dichotomy: the need to assign organisms

as mixotrophs within plankton functional types. Journal of Plankton Research 35, 3–11 (2013).

Geider, R. J., MacIntyre, H. L. & Kana, T. M. A dynamic model of photoadaptation in phytoplankton. Limnology and Oceanography 41, 1–15 (1996).

Grasshoff, K. K. and Ehrhardt, M. Methods of Seawater Analysis. Wiley-VCH, Weinheim (1999)

Guillard, R. R. L. and C. J. Lorenzen. Yellow-green algae with chlorophyllide c. Journal of Phycology 8, 10-14 (1972)

Hartmann, M. et al. Mixotrophic basis of Atlantic oligotrophic ecosystems. Proceedings of the National Academy of Sciences 109, 5756–5760 (2012).

Hillebrand, H., Dürselen, C.-D., Kirschtel, D., Pollingher, U. & Zohary, T. Biovolume calculation for pelagic and benthic microalgae. Journal of phycology 35, 403–424 (1999).

Holen, D. A. Effects of prey abundance and light intensity on the mixotrophic chrysophyte Poterioochromonas malhamensis from a mesotrophic lake. Freshwater Biology 42, 445–455 (1999).

Holen, D. A., Boraas, M. E. Mixotrophy in chrysophytes. In: Chrysophyte Algae: Ecology, Phylogeny and Development (Sandrgren, C. D., Smol, J. P., Kristiansen, J.) 119–140. Cambridge University Press, Cambridge, UK.

Holm, N. P. & Armstrong, D. E. Role of nutrient limitation and competition in controlling the populations of Asterionella formosa and Microcystis aeruginosa in semicontinuous culture1. Limnology and Oceanography 26, 622–634 (1981).

Jones, H. L. J. A classification of mixotrophic protists based on their behaviour. Freshwater Biology **37**, 35–43 (1997).

Jones, R. I. Mixotrophy in planktonic protists: an overview. Freshwater Biology 45, 219–226 (2000).

Katechakis, A., Haseneder, T., Kling, R. & Stibor, H. Mixotrophic versus photoautotrophic specialist algae as food for zooplankton: The light: nutrient hypothesis might not hold for mixotrophs. Limnology and oceanography 50, 1290–1299 (2005).

Katechakis, A. & Stibor, H. The mixotroph Ochromonas tuberculata may invade and suppress specialist phago- and phototroph plankton communities depending on nutrient conditions. Oecologia 148, 692–701 (2006).

Klausmeier, C. A., Litchman, E., Daufresne, T. & Levin, S. A. Phytoplankton stoichiometry. Ecological Research 23, 479–485 (2008).

Litchman, E. & Klausmeier, C. A. Trait-Based Community Ecology of Phytoplankton. Annual Review of Ecology, Evolution, and Systematics 39, 615–639 (2008).

Loladze, I. & Elser, J. J. The origins of the Redfield nitrogen-to-phosphorus ratio are in a homoeostatic protein-to-rRNA ratio: The origins of the Redfield N:P ratio. Ecology Letters 14, 244–250 (2011).

Mitra, A. et al. The role of mixotrophic protists in the biological carbon pump. Biogeosciences 11,

995–1005 (2014).

Nygaard, K., Tobiesen, A. A Survival Strategy During Nutrient Limitation. Limnology and Oceanography 38, 273–279 (1993)

Pålsson, C. & Granéli, W. Diurnal and seasonal variations in grazing by bacterivorous mixotrophs in an oligotrophic clearwater lake. Archiv für Hydrobiologie 157, 289–307 (2003).

Pålsson, C., Daniel, C. Effects of prey abundance and light intensity on nutrition of a mixotrophic flagellate and ist competitive relationship with an obligate heterotroph. Aquatic Microbial Ecology 36, 247–256 (2004)

Ptacnik, R. Omnivory in planktonic food webs: a study on the impact of mixotrophic flagellates and microzooplankton on food web dynamics and productivity. (Christian-Albrechts-Universität, 2003). at http://eprints.uni-kiel.de/1855/1/IFM-BER_328.pdf

Ptacnik, R., Andersen, T., Tamminen, T. Performance of the Redfield Ratio and Family of Nutrient Limitation Indicators as Thresholds for Phytoplankton N vs. P Limitation. Ecosystems 13, 1201–1214 (2010)

Raven, J. A. Phagotrophy in phototrophs. Limnology and Oceanography 42, 198–205 (1997).

Raven, J. A., Beardall, J., Flynn, K. J. & Maberly, S. C. Phagotrophy in the origins of photosynthesis in eukaryotes and as a complementary mode of nutrition in phototrophs: relation to Darwin's insectivorous plants. Journal of Experimental Botany 60, 3975–3987 (2009).

Rothhaupt, K. O. Utilization of Substitutable Carbon and Phosphorus Sources by the Mixotrophic Chrysophyte Ochromonas Sp. Ecology 77, 706 (1996).

Rothhaupt, K. O. Laboratorary Experiments with a Mixotrophic Chrysophyte and Obligately Phagotrophic and Photographic Competitors. Ecology 77, 716 (1996).

Rothhaupt, K.O Nutrient turnover by freshwater bacterivorous flagellates: differences between a heterotrophic and a mixotrophic chrysophyte. Aquatic Microbial Ecology **1**2, 65–70 (1997)

Rottberger, J., Gruber, A., Boenigk, J. & Kroth, P. Influence of nutrients and light on autotrophic, mixotrophic and heterotrophic freshwater chrysophytes. Aquatic Microbial Ecology 71, 179–191 (2013).

Sakshaug, E., Andresen, K., Kiefer, D. A steady state description of growth and light absorption in the marine planktonic diatom Skeletonema costatum. Limnology and Oceanography 34, 198–205 (1989)

Sanders, R. W., Porter, K. G. & Bennett, S. J. Heterotrophic, autotrophic, and mixotrophic nanoflagellates: seasonal abundances and bacterivory in a eutrophic lake. Limnology and Oceanography 35, 1821–1832 (1990).

Sterner, R. W. and Elser J. H. Ecological stoichiometry: the biology of elements from molecules to the biosphere. Princeton University Press. 2002

Štrojsová, A., Vrba, J., Nedoma, J., Komárková, J., Znachor P. Seasonal study of extracellular phosphatase expression in the phytoplankton of a eutrophic reservoir. European Journal of Phycology, 38, 295–306 (2003)

Talling, J. F. Photosynthetic Characteristics of Some Freshwater Plankton Diatoms in Relation to Underwater Radiation. New Phytologist 56, 29–50 (1957)

Thingstad, T. F., Havskum, H., Garde, K. & Riemann, B. On the Strategy of 'Eating Your Competitor': A Mathematical Analysis of Algal Mixotrophy. Ecology 77, 2108 (1996).

Tittel, J. et al. Mixotrophs combine resource use to outcompete specialists: implications for aquatic food webs. Proceedings of the National Academy of Sciences 100, 12776–12781 (2003).

Unrein, F., Gasol, J. M., Not, F., Forn, I. & Massana, R. Mixotrophic haptophytes are key bacterial grazers in oligotrophic coastal waters. The ISME Journal 8, 164–176 (2014).

Urabe, J., Gurung, T. B., Yoshida, T. Effects of phosphorus supply on phagotrophy by the mixotrophic alga Uroglena americana (Chrysophyceae). Aquatic Microbial Ecology 18, 77-83 (1999)

Winter, C., Kerros, M.-E. & Weinbauer, M. G. Seasonal and depth-related dynamics of prokaryotes and viruses in surface and deep waters of the northwestern Mediterranean Sea. Deep Sea Research Part I: Oceanographic Research Papers 56, 1972–1982 (2009).

Wood, S. N. Thin plate regression splines. Journal of the Royal Statistical Society: Series B (Statistical Methotology) 65, Part 1, 95–114 (2003)

Zonneveld, C. A cell-based model for the chlorophyll a to carbon ratio in phytoplankton. Ecological Modelling 113, 55–70 (1998).

Zubkov, M. V. & Tarran, G. A. High bacterivory by the smallest phytoplankton in the North Atlantic Ocean. Nature 455, 224–226 (2008).

# Abstract

Mixotrophic algae have to maintain energy costly cell structures for photosynthesis and phagotrophic nutrition. Such additional expanses accompanied by reduced efficiency could result in overall higher light requirement and/or lower maximum growth rates. In this study the growth rates of three different mixotrophic species, *Poterioochromonas malhamensis*, *Ochromonas tuberculata* and *Dinobryon divergens*, were studied under different light conditions. Algae were grown as monocultures in batch experiments on WC medium. By comparing data from the literature, a clear tendency was found that mixotrophs exhibit lower maximum growth rates than photoautotrophic algae.

Glucose addition was used to trigger bacterial growth and resulted in higher growth rates of the mixotrophic algae. Effects of glucose addition at low light were species specific and did not support growth of *D. divergens*. Obviously *D. divergens* cannot sufficiently compensate unmet energy demands by phagotrophy. On the contrary, *P. malhamensis* mainly gains energy by ingesting bacteria and uses light as a source of energy only when prey is limiting.

Knowing to what extent a mixotrophic species depends on light and bacteria, allows for more precise predictions where its ecological niche is located in the water column. It also allows for making assumptions on how these algae cope when competing with other species, e.g. phototrophic phytoplankton.

In an additional experiment, communities of the mixotrophic *Dinobryon divergens*, the photoautotrophic *Asterionella formosa* and the heterotrophic nanoflagellate *Spumella sp*. were exposed to a light gradient. When light intensity was too low, *Asterionella* was able to outcompete *Dinobryon*, whereas under intermediate and high light levels species coexisted. Presence of *Dinobryon* clearly had an effect on bacteria populations, which was also dependent on light intensity. When enough light was available *Dinobryon* populations increased and thus reduced bacteria density.

# Zusammenfassung

Mixotrophe Algen müssen Zellstrukturen sowohl für die Photosynthese als auch für die phagotrophe Ernährung erhalten, was viel Energie benötigt. Diese zusätzlichen „Kosten“, zusammen mit einer weniger effizienten Nutzung der Ressourcen, kann zu einem höheren Bedarf an Licht und/oder niedrigeren maximalen Wachstumsraten führen. In dieser Studie wurden die Wachstumsraten der drei mixotrophen Arten *Poterioochromonas malhamensis*, *Ochromonas tuberculata* und *Dinobryon divergens* bei unterschiedlichen Lichtverhältnissen bestimmt. Die Algen wurden als Monokulturen in einem Batch-Versuch auf WC-Medium kultiviert. Beim Vergleich der Daten mit der Literatur wurde festgestellt, dass Mixotrophe tatsächlich niedrigere Wachstumsraten aufweisen als photoautotrophe Arten.

In einer zweiten Versuchsreihe wurde das bakterielle Wachstum durch die Zugabe von Glukose angeregt, was höhere Wachstumsraten der mixotrophen Algen zur Folge hatte. Bei geringer Lichtintensität war dieser Effekt artspezifisch. *Dinobryon divergens* wurde bei zu wenig Licht nicht durch höhere Bakteriendichte zu schnellerem Wachstum angeregt, da diese Art offensichtlich ihren Energiebedarf nicht durch Phagotrophie decken kann. Im Gegensatz dazu bezieht *P. malhamensis* Energie vor allem durch die Ingestion von Bakterien und nutzt Licht nur bei geringer Bakteriendichte als Energiequelle.

Das Wissen um die Abhängigkeit einer Art von Licht und Bakterien erlaubt genauere Aussagen über die ökologische Nische einer Art und in welcher Tiefe der Wassersäule sie vermehrt zu finden ist. Auch wird es einfacher, das Verhalten der Algenpopulationen vorherzusagen, wenn Mixotrophe mit anderen Arten, beispielsweise Photoautotrophen, in Konkurrenz stehen.

In einem weiteren Experiment wurden Artengemeinschaften aus der mixotrophen Alge *Dinobryon divergens*, der photoautotrophen Art *Asterionella formosa* und dem heterotrophen Nanoflagellaten *Spumella sp*. einem Lichtgradient ausgesetzt. Bei niedriger Lichtintensität war *Asterionella formosa* die dominierende Art, während sie bei mittlerer und hoher Lichtintensität mit *Dinobryon divergens* koexistierte. Die Anwesenheit von *Dinoryon* hatte einen deutlichen aber von der Lichtintensität abhängigen Effekt auf die Bakterienpopulationen. War genügend Licht vorhanden, erreichte die *Dinobryon*-Population eine höhere Dichte, was zu einer niedrigeren Bakterienabundanz führte.

# Tables



# Figures

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# Curriculum Vitae

## Education

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| 03/2012 – 10/2015 | Master studies of Ecology at the University of Vienna – Department of Limnology and Bio-Oceanography |
| 09/2012 – 01/2013 | Erasmus Semester in Madrid, Spain, at the Universidad Autónoma de Madrid |
| 10/2008 – 02/2012 | Bachelor studies of Biology at the University of Vienna – Specialization: Ecology |
| 09/2005 – 06/2006 | College of Hospitality Management at the College of Tourism, Klessheim |
| 09/1997 – 06/2005 | Secondary School, Privatgymnasium St. Ursula, Salzburg |

## Work Experience

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| 06/2014 | Tutor at the Department of Limnology and Bio-Oceanography, University of Vienna, for the practical course “Biogeochemistry of streams and lakes” |
| 06/2013 – 07/2013 | FemTech internship at Wassercluster Lunz (Working Group AQUASCALE) |
| 08/2012 – 09/2012 | FemTech internship at the department of Soil Ecology, Bundesamt für Wald, Vienna |
| Since 2008 | part time jobs as restaurant service employee in Vienna |
| 2006 – 2008 | Restaurant service employee in Vienna and Salzburg |

## Conferences

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| 04/2015 | Poster presentation at the conference “Fresh Blood for Fresh Water” in Mondsee, Austria |

## Commendations

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| 10/2013 | academic achievement scholarship of the University of Vienna |
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## Further Qualifications

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| Computer Skills | MS-Office |
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| Languages | German (first language) |
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